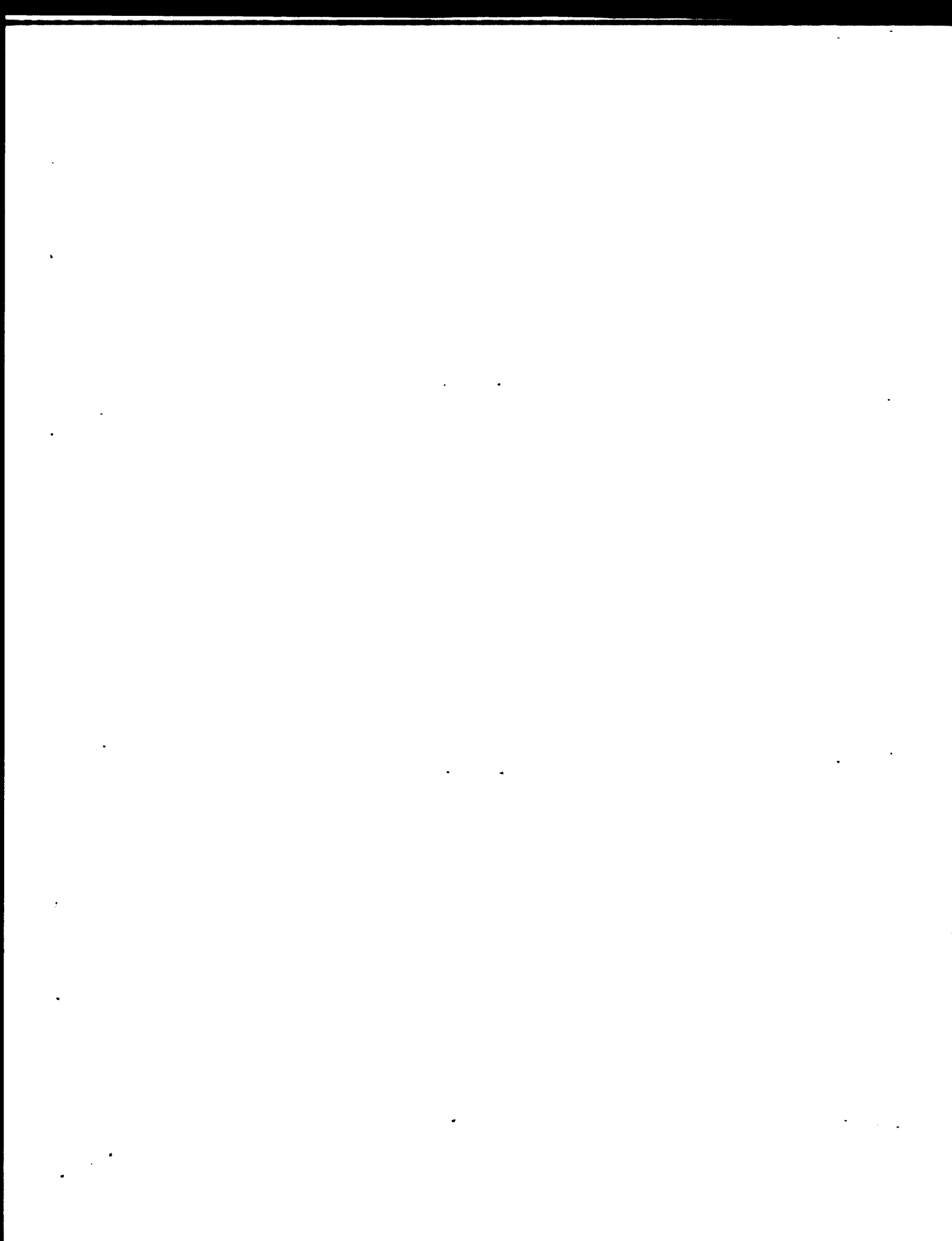


PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

| | | |
|---|-----------|--|
| (51) International Patent Classification ⁷ : A61K 31/00 | A2 | (11) International Publication Number: WO 00/15211 (43) International Publication Date: 23 March 2000 (23.03.00) |
| (21) International Application Number: PCT/US99/21377 (22) International Filing Date: 17 September 1999 (17.09.99) (30) Priority Data: 09/156,102 17 September 1998 (17.09.98) US 60/126,489 26 March 1999 (26.03.99) US <i>17 Mar 01 / 30 Mar 99</i> (71) Applicant (for all designated States except US): AKESIS PHARMACEUTICALS, INC. [US/US]; Suite B, 10565 Science Center Drive, San Diego, CA 92121 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): FINE, Stuart [US/US]; 2230 White Oak Drive, Northbrook, IL 60062 (US). KINSELLA, Kevin [US/US]; 1735 Castellana Road, La Jolla, CA 92037 (US). (74) Agents: TAFT, Kingsley, L. et al.; Foley, Hoag & Eliot, LLP, One Post Office Square, Boston, MA 02109 (US). | | (81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>Without international search report and to be republished upon receipt of that report.</i> |
| (54) Title: COMPOSITIONS AND METHODS FOR TREATMENT OF GLUCOSE METABOLISM DISORDERS (57) Abstract Compositions and methods of using the same for the treatment of diabetes and other disorders of glucose metabolism are provided. Compositions may include an anti-diabetic agent and one or more of a bioavailable source of chromium and vanadium. | | |



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| | | |
|--|-----------|---|
| (51) International Patent Classification ⁶ : C12N 15/51, 7/01, C07K 14/02, 16/08, C12Q 1/68, A61K 39/29 | A1 | (11) International Publication Number: WO 99/66048 (43) International Publication Date: 23 December 1999 (23.12.99) |
| (21) International Application Number: PCT/SG98/00046 (22) International Filing Date: 19 June 1998 (19.06.98) (71) Applicant (for all designated States except US): GOVERNMENT OF REPUBLIC OF SINGAPORE [SG/SG]; Ministry of Health, College of Medicine Building, 18 College Road, Singapore 169854 (SG). (72) Inventors; and (75) Inventors/Applicants (for US only): OON, Chong, Jin [SG/SG]; 14A Princess of Wales Road, Singapore 266914 (SG). LIM, Gek, Keow [SG/SG]; 16 Telok Kurau, Lorong G, Singapore 426180 (SG). ZHAO, Yi [SG/SG]; Block 345, #12-270 Bukit Batok Street 34, Singapore 650345 (SG). CHEN, Wei, Ning [SG/SG]; Block 104, #22-114 Spottiswoode Park Road, Singapore 080104 (SG). (74) Agent: DREW & NAPIER; 20 Raffles Place, #17-00 Ocean Towers, Singapore 048620 (SG). | | (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> |
| (54) Title: A MUTANT HUMAN HEPATITIS B VIRAL STRAIN AND USES THEREOF | | |
| (57) Abstract <p>This invention provides an isolated strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-133 Oon Strain (Methionine to Threonine) which constituent viral genome is deposited under Accession Nos. P97121501, P97121502 and P97121503 with the European Collection of Cell Culture on 15th December 1997. This invention also provides an isolated nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine rather than a methionine. This invention also provides an isolated nucleic acid which encodes a peptide, wherein the peptide is encoded by a nucleic acid molecule comprising nucleotides 527 through 595 of SEQ. ID. No. 1 and the purified peptide. This invention also provides various methods of using the disclosed isolated nucleic acid and peptides. This invention further provides various uses of the disclosed isolated nucleic acid, polypeptides and peptides, and antibodies.</p> | | |

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(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
1 February 2001 (01.02.2001)

PCT

(10) International Publication Number
WO 01/07652 A2

- (51) International Patent Classification⁷: **C12Q 1/68**
- (21) International Application Number: **PCT/IB00/01079**
- (22) International Filing Date: **28 July 2000 (28.07.2000)**
- (25) Filing Language: **English**
- (26) Publication Language: **English**
- (30) Priority Data:
09/362,394 **28 July 1999 (28.07.1999)** **US**
- (71) Applicant: **THE GOVERNMENT OF THE REPUBLIC OF SINGAPORE** [SG/SG]; Attorney General's Chambers, 1 Coleman Street #10-00, Singapore 179803 (SG).
- (72) Inventors: **OON, Chong-Jin**; 14A Princess of Wales Road, Singapore 266914 (SG). **CHEN, Wei-Ning**; Blk 104, Spottiswoode Park Road, #22-114, Singapore 080104 (SG). **LEONG, Ai-Lin**; Blk. 263, Yishun Street 22, #12-161, Singapore 760263 (SG). **KOH, Shiuan**; Blk. 206, Toa Payoh North, #02-1199, Singapore 310206 (SG).
- (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.
- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
- Published:**
- *Without international search report and to be republished upon receipt of that report.*
- For two letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*



WO 01/07652 A2

(54) Title: DETECTION OF HUMAN HEPATITIS B VIRUS SURFACE ANTIGEN MUTANTS BY SPECIFIC AMPLIFICATION AND ITS APPLICATION ON GENE CHIP

(57) Abstract: Novel DNA probe sequences for detection, by polymerase chain reaction, of human hepatitis B virus surface antigen mutant 145 (Glycine to Arginine) from serum samples. As a direct application, these specific DNA probes are immobilized on solid glass supports (gene chip) for detection of human hepatitis B virus surface antigen mutant 145 (Glycine to Arginine) by fluorescence.

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Our Reference CG/PHM/PAT/8013374

Your Reference --

Date 18 March 2000

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as the International Preliminary Examining Authority
D-80298 Munich
GERMANY

Via Facsimile/By Post
Fax No.: 012-49-89-2399-4465
No. of Pages: 4 only

Attn: Von Kempis B G M

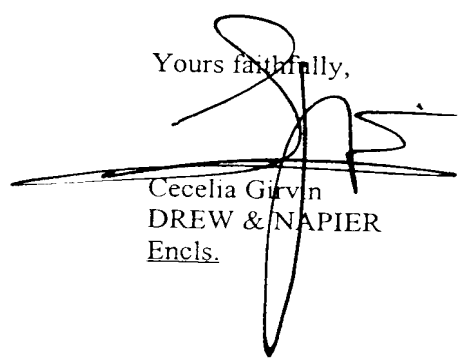
Dear Sirs,

Demand under Article 31 of the Patent Cooperation Treaty
PCT International Application No. PCT/SG98/00045
Entitled: A Vaccine-Induced Hepatitis B Viral Strain and Uses Thereof
Applicant: Government of the Republic of Singapore

We draw your attention to an obvious clerical error in Box V on page 3 of the PCT Demand under Chapter II (Form PCT/IPEA/401). We had erroneously indicated "AE" (United Arab Emirates) as an excluded State when it should have been elected. We refer to our letter dated 18 January 2000 and the attached Annex A which indicates the states that the applicant wishes to elect. Annex A clearly reflected United Arab Emirates (AE) as an elected State. Copies of the relevant pages of our Demand under Article 31 of the PCT is highlighted and **enclosed herewith for your easy reference.

We would appreciate if the EPO would correct our application to include United Arab Emirates as an elected state as soon as possible.

Yours faithfully,


Cecilia Girvin
DREW & NAPIER
Encls.

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Our Reference CG/PHM/PAT/8013374

Direct Dial

Your Reference --

Date 18 January 2000

European Patent Office
as the International Preliminary Examining Authority
D-80298 Munchen
GERMANY

Via Facsimile/Confirmation by Courier
Fax No.: 012-4989-2399-4465
No. of Pages: 7 only

Dear Sirs,

Demand under Article 31 of the Patent Cooperation Treaty
PCT International Application No. PCT/SG98/00045
Entitled: A Vaccine-Induced Hepatitis B Viral Strain and Uses Thereof
Applicant: Government of the Republic of Singapore

DEMAND FOR INTERNATIONAL PRELIMINARY EXAMINATION

Our client has instructed us to file a Demand for the International Preliminary Examination. In this respect, the undersigned respectfully requests that the international application specified above be the subject of International Preliminary Examination according to the PCT. We submit herewith the following:-

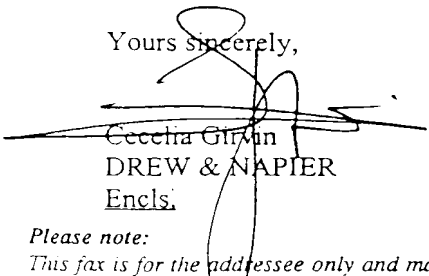
1. The PCT Demand;
2. The Fee Calculation Sheet; and
3. Our cheque for the amount of EUR\$1,681.00.



Our client wishes to elect the states as mentioned in the Annex A **attached.

We thank you for your attention in this matter and we look forward to receiving a favourable International Preliminary Examination Report.

Yours sincerely,


Cecilia Girvin
DREW & NAPIER
Encls.

Please note:

This fax is for the addressee only and may contain confidential information and/or may be subject to legal privilege. If it has reached you in error please inform us immediately on telephone numbers (65) 531 2295 or (65) 531 2488, reverse charges if necessary.

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Annex A

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Austria
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South Africa
Spain
Sri Lanka
Sweden
Trinidad and Tobago
Turkey
Ukraine
United Arab Emirates
United Kingdom
United States of America
Vietnam
Yugoslavia





Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The following person is ☒ agent ☐ common representative
 and ☒ has been appointed earlier and represents the applicant(s) also for international preliminary examination.
☐ is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.
☐ is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.

Name and address: (Family name followed by given name, for a legal entity, full official designation.
 The address must include postal code and name of country.)

CECELIA GIRVIN ; JUPITER KONG
 DREW AND NAPIER
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☒ Address for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION

Statement concerning amendments:*

1. The applicant wishes the international preliminary examination to start on the basis of:

☒ the international application as originally filed

the description ☐ as originally filed
☐ as amended under Article 34

the claims ☐ as originally filed
☐ as amended under Article 19 (together with any accompanying statement)
☐ as amended under Article 34

the drawings ☐ as originally filed
☐ as amended under Article 34

2. ☐ The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.

3. ☐ The applicant wishes the start of the international preliminary examination to be postponed until the expiration of 20 months from the priority date unless the International Preliminary Examining Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69.1(d)). (This check-box may be marked only where the time limit under Article 19 has not yet expired.)

* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Language for the purposes of international preliminary examination: ENGLISH

☒ which is the language in which the international application was filed.

☐ which is the language of a translation furnished for the purposes of international search.

☒ which is the language of publication of the international application.

☐ which is the language of the translation (to be) furnished for the purposes of international preliminary examination.

Box No. V ELECTION OF STATES

The applicant hereby elects all eligible States (that is, all States which have been designated and which are bound by Chapter II of the PCT)

excluding the following States which the applicant wishes not to elect: ~~AE~~, AL, AM, AR, BA, BY, CH, CZ, DE, DK, EE, EG, ES, FI, FR, GB, GR, HU, IL, IN, JP, KR, LC, LR, LU, LT, LV, MD, MG, MK, MN, MW, NO, NZ, PL, PT, RO, RU, SE, SI, SK, SL, TJ, TM, TR, UA, UZ, ZW



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Our Reference CG/PHM/PAT/8013374

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Date 18 March 2000

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Via Facsimile/Confirmation by Post
Fax No.: 012-41-22-740-1435
No. of Pages: 4 only

Attn: F. Baechler

Dear Sirs,

Demand under Article 31 of the Patent Cooperation Treaty
PCT International Application No. PCT/SG98/00045
Entitled: A Vaccine-Induced Hepatitis B Viral Strain and Uses Thereof
Applicant: Government of the Republic of Singapore

Thank you for the Information Concerning Elected Offices Notified of Their Election (Form PCT/IB332).

Upon review of Form PCT/IB332, we note that four of the elected states namely Croatia, India, South Africa and United Arab Emirates are omitted.

We also draw your attention to an obvious clerical error in Box V on page 3 of the PCT Demand under Chapter II (Form PCT/IPEA/401). We had erroneously indicated "AE" (United Arab Emirates) as an excluded State when it should have been elected. We refer to our letter dated 18 January 2000 and the attached Annex A which indicates the states that the applicant wishes to elect. Annex A clearly reflected United Arab Emirates (AE) as an elected State. Copies of the relevant pages of our Demand under Article 31 of the PCT is highlighted and **enclosed herewith for your easy reference.

We would appreciate if The International Bureau would issue a corrected Form PCT/IB/332 to include Croatia, India, South Africa and United Arab Emirates as elected States as soon as possible.

Yours faithfully,

Cecilia Girvin
DREW & NAPIER
Encls.

Managing Partner
Davinder Singh,
Senior Counsel

Senior Consultant
J Grimberg,
Senior Counsel

Partners
R Raj Singam
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Ian Koh
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Jean Thio
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Werner Tsu
Shamaine Lim

Senior International Lawyer
John K Moline (WI, USA)

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Regional Desks: Greater China, India, Indonesia, Malaysia & Vietnam



DREW & NAPIER

Estd 1889

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Corporate/Tax
(65) 535 1952 Conveyancing
(65) 533 0694 Intellectual
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(65) 532 7149 Litigation/Shipping
(65) 533 0693 Litigation/Shipping

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(Malaysia) Sdn Bhd
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Facsimile (03) 2162 2804
- Drew & Napier, Vietnam Branch
(Hanoi)
Telephone (844) 514 1995/1996
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office hours & on holidays:
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Important Notice: Service of Court documents by fax is not accepted

E-mail: mail@drew-and-napier.com.sg

Direct E-mail: cecilia.girvin@drew-and-napier.com.sg

Our Reference CG/PHM/PAT/8013374

Direct Dial

Your Reference --

Date 18 January 2000

European Patent Office
as the International Preliminary Examining Authority
D-80298 Munchen
GERMANY

Via Facsimile/Confirmation by Courier
Fax No.: 012-4989-2399-4465
No. of Pages: 7 only


Dear Sirs,

Demand under Article 31 of the Patent Cooperation Treaty
PCT International Application No. PCT/SG98/00045
Entitled: A Vaccine-Induced Hepatitis B Viral Strain and Uses Thereof
Applicant: Government of the Republic of Singapore

DEMAND FOR INTERNATIONAL PRELIMINARY EXAMINATION

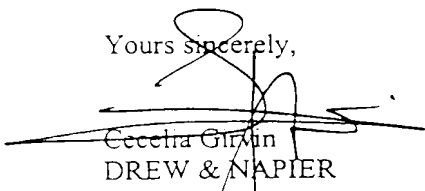
Our client has instructed us to file a Demand for the International Preliminary Examination. In this respect, the undersigned respectfully requests that the international application specified above be the subject of International Preliminary Examination according to the PCT. We submit herewith the following:-

1. The PCT Demand;
2. The Fee Calculation Sheet; and
3. Our cheque for the amount of EUR\$1,681.00.

 Our client wishes to elect the states as mentioned in the Annex A **attached.

We thank you for your attention in this matter and we look forward to receiving a favourable International Preliminary Examination Report.

Yours sincerely,


Cecilia Girvin
DREW & NAPIER
Encls.

Please note:

This fax is for the addressee only and may contain confidential information and/or may be subject to legal privilege. If it has reached you in error, please inform us immediately on telephone numbers (65) 531 2295 or (65) 531 2488, reverse charges if necessary.

Managing Partner
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Senior Consultant
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Carole Gaud (France)

Regional Desks: Greater China, India, Indonesia, Malaysia & Vietnam



Annex A

Australia
Austria
Barbados
Belgium
Brazil
Bulgaria
Canada
China
Croatia
Denmark
Finland
France
Germany
Greece
Hungary
Iceland
India
Indonesia
Ireland
Israel
Italy
Japan
Kenya
Luxembourg
Mexico
Netherlands
New Zealand
Norway
Poland
Portugal
Republic of Korea
Russia Federation
Singapore
South Africa
Spain
Sri Lanka
Sweden
Trinidad and Tobago
Turkey
Ukraine
United Arab Emirates
United Kingdom
United States of America
Vietnam
Yugoslavia





Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCEThe following person is ☒ agent ☐ common representativeand ☒ has been appointed earlier and represents the applicant(s) also for international preliminary examination.☐ is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.☐ is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.Name and address: *(Family name followed by given name, for a legal entity, full official designation. The address must include postal code and name of country.)*CECELIA GIRVIN ; JUPITER KONG
DREW AND NAPIER
20 RAFFLES PLACE
#17-00 OCEAN TOWERS
SINGAPORE 048620

Telephone No.:

(65) 535 0733

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(65) 533 0694

☒ Address for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.**Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION****Statement concerning amendments:***

1. The applicant wishes the international preliminary examination to start on the basis of:

☒ the international application as originally filedthe description ☐ as originally filed☐ as amended under Article 34the claims ☐ as originally filed☐ as amended under Article 19 (together with any accompanying statement)☐ as amended under Article 34the drawings ☐ as originally filed☐ as amended under Article 342. ☐ The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.3. ☐ The applicant wishes the start of the international preliminary examination to be postponed until the expiration of 20 months from the priority date unless the International Preliminary Examining Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69 1(d)). *(This check-box may be marked only where the time limit under Article 19 has not yet expired.)*

• Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Language for the purposes of international preliminary examination: ENGLISH☒ which is the language in which the international application was filed.☐ which is the language of a translation furnished for the purposes of international search.☒ which is the language of publication of the international application.☐ which is the language of the translation (to be) furnished for the purposes of international preliminary examination.**Box No. V ELECTION OF STATES**The applicant hereby elects all eligible States *(that is, all States which have been designated and which are bound by Chapter II of the PCT)*excluding the following States which the applicant wishes not to elect: ~~AE, AL, AM, AR, BA, BY, CH, CL, CN, CZ, EE, GD, GE, GR, GM, KG, KP, KR, LC, LR, LS, LT, LV, MD, MG, MK, MN, MW, RO, SD, SI, SK, SL, TJ, TM, US, UZ, VN~~



DREW & NAPIER

Estd 1889

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Trademark & Patent Agents

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Corporate Tax
(65) 535 1952 Conveyancing
(65) 533 0694 Intellectual
Property
(65) 532 7149 Litigation, Shipping
(65) 533 0693 Litigation, Shipping

09 / 710533
Regional Offices:
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Drewmarks Patents & Designs
(Malaysia) Sdn Bhd
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Facsimile (03) 2162 2804
• Drew & Napier, Vietnam Branch
(Hanoi)
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Our Reference CG/PHM/PAT/8013374

Direct Dial

Your Reference --

Date 18 January 2000

European Patent Office
as the International Preliminary Examining Authority
D-80298 Munchen
GERMANY

Via Facsimile/Confirmation by Courier
Fax No.: 012-4989-2399-4465
No. of Pages: 7 only

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Applicant: Government of the Republic of Singapore

DEMAND FOR INTERNATIONAL PRELIMINARY EXAMINATION

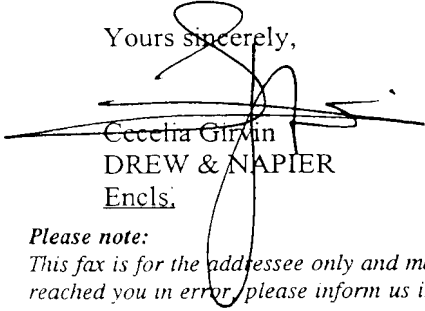
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1. The PCT Demand;
2. The Fee Calculation Sheet; and
3. Our cheque for the amount of EUR\$1,681.00.

Our client wishes to elect the states as mentioned in the Annex A **attached.

We thank you for your attention in this matter and we look forward to receiving a favourable International Preliminary Examination Report.

Yours sincerely,


Cecelia Girvin
DREW & NAPIER
Encls.

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Foreign Lawyers
John K Moline (WI, USA)
Grace Ho (NSW, Austl)
Carole Gaud (France)

Regional Desks: Greater China, India, Indonesia, Malaysia & Vietnam



Annex A

Australia
Austria
Barbados
Belgium
Brazil
Bulgaria
Canada
China
Croatia
Denmark
Finland
France
Germany
Greece
Hungary
Iceland
India
Indonesia
Ireland
Israel
Italy
Japan
Kenya
Luxembourg
Mexico
Netherlands
New Zealand
Norway
Poland
Portugal
Republic of Korea
Russia Federation
Singapore
South Africa
Spain
Sri Lanka
Sweden
Trinidad and Tobago
Turkey
Ukraine
United Arab Emirates
United Kingdom
United States of America
Vietnam
Yugoslavia



The demand must be filed directly with the competent International Preliminary Examining Authority or, if two or more Authorities are competent, with the one chosen by the applicant. The full name or two-letter code of that Authority may be indicated by the applicant on the line below:

IPEA/ EP

PCT

CHAPTER II

DEMAND

under Article 31 of the Patent Cooperation Treaty:

The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty and hereby elects all eligible States (except where otherwise indicated).

| | |
|--|--|
| For International Preliminary Examining Authority use only | |
| Identification of IPEA | Date of receipt of DEMAND |
| Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION | |
| Applicant's or agent's file reference CG 1P4M/PAT/8013374 | |
| International application No. PCT/SG98/00045 | International filing date (day/month/year) 19 JUNE 1998 |
| (Earliest) Priority date (day/month/year) - | |
| Title of invention A VACCINE-INDUCED HEPATITIS B VIRAL STRAIN AND USES THEREOF | |
| Box No. II APPLICANT(S) | |
| Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.) | |
| GOVERNMENT OF REPUBLIC OF SINGAPORE MINISTRY OF HEALTH COLLEGE OF MEDICINE BUILDING 18 COLLEGE ROAD SINGAPORE 169854 | |
| Telephone No.: (65) 325 9079 | |
| Facsimile No.: (65) 325 9211 | |
| Teleprinter No.: | |
| State (that is, country) of nationality: N.A. | State (that is, country) of residence: N.A. |
| Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.) | |
| DR. OON CHONG JIN 14A PRINCESS OF WALES ROAD SINGAPORE 266914 | |
| State (that is, country) of nationality: SINGAPORE | State (that is, country) of residence: SINGAPORE |
| Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.) | |
| MDM LIM GEK KEOW 16 TELOK KURAU LOHONG G SINGAPORE 426180 | |
| State (that is, country) of nationality: SINGAPORE | State (that is, country) of residence: SINGAPORE |
| <input checked="" type="checkbox"/> Further applicants are indicated on a continuation sheet. | |

Continuation of Box No. II APPLICANT(S)

*If none of the following sub-boxes is used, this sheet should not be included in the demand.*Name and address: *(Family name followed by given name, for a legal entity, full official designation. The address must include postal code and name of country.)*

DR. ZHAO YI
BLOCK 345, #12-270
BUKIT BATOK STREET 34
SINGAPORE 650345

State *(that is, country)* of nationality:

SINGAPORE

State *(that is, country)* of residence:

SINGAPORE

Name and address: *(Family name followed by given name, for a legal entity, full official designation. The address must include postal code and name of country.)*

DR. CHEN WEI NING
BLOCK 104 SPOTTISWOODE PARK ROAD
#22-114
SINGAPORE 080104

State *(that is, country)* of nationality:

SINGAPORE

State *(that is, country)* of residence:

SINGAPORE

Name and address: *(Family name followed by given name, for a legal entity, full official designation. The address must include postal code and name of country.)*

MS. LEONG AI LIN
BLOCK 119, #04-443
TOA PAYOH LORONG 1
SINGAPORE 310119

State *(that is, country)* of nationality:

SINGAPORE

State *(that is, country)* of residence:

SINGAPORE

Name and address: *(Family name followed by given name, for a legal entity, full official designation. The address must include postal code and name of country.)*State *(that is, country)* of nationality:State *(that is, country)* of residence:

Further applicants are indicated on another continuation sheet.



Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCEThe following person is ☒ agent ☐ common representativeand ☒ has been appointed earlier and represents the applicant(s) also for international preliminary examination.☐ is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.☐ is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*CECELIA GIRVIN ; JUPITER KONG
DREW AND NAPIER
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#17-00 OCEAN TOWERS
SINGAPORE 048620

Telephone No.:

(65) 535 0733

Facsimile No.:

(65) 535 4906

Teleprinter No.:

(65) 533 0694

☒ **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.**Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION****Statement concerning amendments:***1. The applicant wishes the international preliminary examination to **start on the basis of:**☒ the international application as originally filedthe description ☐ as originally filed☐ as amended under Article 34the claims ☐ as originally filed☐ as amended under Article 19 (together with any accompanying statement)☐ as amended under Article 34the drawings ☐ as originally filed☐ as amended under Article 342. ☐ The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.3. ☐ The applicant wishes the start of the international preliminary examination **to be postponed** until the expiration of 20 months from the priority date unless the International Preliminary Examining Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69.1(d)). *(This check-box may be marked only where the time limit under Article 19 has not yet expired)*

* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Language for the purposes of international preliminary examination: ENGLISH☒ which is the language in which the international application was filed.☐ which is the language of a translation furnished for the purposes of international search.☒ which is the language of publication of the international application.☐ which is the language of the translation (to be) furnished for the purposes of international preliminary examination.**Box No. V ELECTION OF STATES**The applicant hereby **elects all eligible States** (that is, all States which have been designated and which are bound by Chapter II of the PCT)excluding the following States which the applicant wishes **not to elect**: AE, AL, AM, AZ, BA, BY, CH, CZ, DE, EE, EG, GE, GH, GM, KG, KP, KZ, LC, LR, LS, LT, LV, MD, MG, MK, MN, MW, RO, SD, SE, SK, SL, TJ, TM, UG, UZ, ZW



Box No. VI CHECK LIST

The demand is accompanied by the following elements, in the language referred to in Box No. IV, for the purposes of international preliminary examination:

- | | |
|--|----------|
| 1. translation of international application | sheets |
| 2. amendments under Article 34 | sheets |
| 3. copy (or, where required, translation) of amendments under Article 19 | sheets |
| 4. copy (or, where required, translation) of statement under Article 19 | sheets |
| 5. letter | 1 sheets |
| 6. other (specify) Annex A (Election of States): | 1 sheets |

For International Preliminary Examining Authority use only

| received | not received |
|--------------------------|--------------------------|
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |

The demand is also accompanied by the item(s) marked below:

- | | |
|--|---|
| 1. <input checked="" type="checkbox"/> fee calculation sheet | 4. <input type="checkbox"/> statement explaining lack of signature |
| 2. <input type="checkbox"/> separate signed power of attorney | 5. <input type="checkbox"/> nucleotide and or amino acid sequence listing in computer readable form |
| 3. <input type="checkbox"/> copy of general power of attorney; reference number, if any: | 6. <input type="checkbox"/> other (specify): |

Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).


CECELIA GIRVIN


JUPITER KONG

For International Preliminary Examining Authority use only

1. Date of actual receipt of DEMAND:

2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b):

3. ☐ The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below, does not apply.

☐ The applicant has been informed accordingly.

4. ☐ The date of receipt of the demand is WITHIN the period of 19 months from the priority date as extended by virtue of Rule 80.5.

5. ☐ Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rule 82.

For International Bureau use only

Demand received from IPEA on:



PCT

FEE CALCULATION SHEET

Annex to the Demand for international preliminary examination

| | |
|---|--|
| International application No. PCT/SG98/00045 | For International Preliminary Examining Authority use only |
| Applicant's or agent's file reference CG/PHM/PAT/8013374 | Date stamp of the IPEA |
| Applicant GOVERNMENT OF REPUBLIC OF SINGAPORE | |
| Calculation of prescribed fees <div style="display: flex; justify-content: space-between; align-items: flex-end;"> <div style="width: 60%;"> 1. Preliminary examination fee </div> <div style="width: 35%; text-align: right;"> <div style="border: 1px solid black; padding: 2px 10px;">EUR 1,533</div> <div style="border: 1px solid black; padding: 2px 5px; float: right;">P</div> </div> </div> <div style="display: flex; justify-content: space-between; align-items: flex-end; margin-top: 20px;"> <div style="width: 60%;"> 2. Handling fee <i>(Applicants from certain States are entitled to a reduction of 75% of the handling fee. Where the applicant is (or all applicants are) so entitled, the amount to be entered at H is 25% of the handling fee.)</i> </div> <div style="width: 35%; text-align: right;"> <div style="border: 1px solid black; padding: 2px 10px;">EUR 148</div> <div style="border: 1px solid black; padding: 2px 5px; float: right;">H</div> </div> </div> <div style="display: flex; justify-content: space-between; align-items: flex-end; margin-top: 20px;"> <div style="width: 60%;"> 3. Total of prescribed fees Add the amounts entered at P and H and enter total in the TOTAL box </div> <div style="width: 35%; text-align: right;"> <div style="border: 1px solid black; padding: 2px 10px;">EUR 1681</div> <div style="border: 1px solid black; padding: 2px 10px; margin-top: 5px;">TOTAL</div> </div> </div> | |
| Mode of Payment <div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <input type="checkbox"/> authorization to charge deposit account with the IPEA (see below) </div> <div style="width: 45%;"> <input type="checkbox"/> cash </div> </div> <div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <input checked="" type="checkbox"/> cheque </div> <div style="width: 45%;"> <input type="checkbox"/> revenue stamps </div> </div> <div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <input type="checkbox"/> postal money order </div> <div style="width: 45%;"> <input type="checkbox"/> coupons </div> </div> <div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <input type="checkbox"/> bank draft </div> <div style="width: 45%;"> <input type="checkbox"/> other (specify) </div> </div> | |
| Deposit Account Authorization <i>(this mode of payment may not be available at all IPEAs)</i> The IPEA/ _____ <input type="checkbox"/> is hereby authorized to charge the total fees indicated above to my deposit account. <input type="checkbox"/> <i>(this check-box may be marked only if the conditions for deposit accounts of the IPEA so permit)</i> is hereby authorized to charge any deficiency or credit any overpayment in the total fees indicated above to my deposit account. | |
| Deposit Account Number _____ | Date (day/month/year) 18 January 2000 |
| Signature CECELIA GIRVIN/JUPITER KONG | |



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Our Reference MJ/TSF/vc/PAT/8013374

Your Reference --

Date 9 June 2000

European Patent Office
As the International Preliminary Examining Authority
D-80298 Munich
GERMANY

Attn : Scheffzyk, I

Dear Sirs,

Demand under Article 31 of the Patent Cooperation Treaty
PCT International Application No. PCT/SG98/00045
Entitled : A Vaccine-Induced Hepatitis B Viral Strain and Uses Thereof
Applicant : Government of the Republic of Singapore

Via Facsimile/By Courier
Fax No. 012-49-89-2399-4465
No. of Page(s) : 29 only

**IMMEDIATE
ATTENTION**

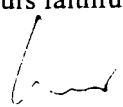
Thank you for the Written Opinion dated 10 March 2000 issued in connection with the International Preliminary Examination Report.

Our client wishes to file arguments and amendments in response to the Written Opinion dated 10 March 2000. In accordance with Rule 66.3, we submit herewith the following:-

- (i) A copy of replacement sheets (pages 35-51), and
- (ii) An annex stating the applicant's arguments on response to the Written Opinion.

We look forward to receiving a favourable Examination Report as soon as possible.

Yours faithfully,


Morris John
Drew & Napier
Encls.

Please note:

This communication is for the addressee only and may contain confidential information and/or may be subject to legal privilege. If it has reached you in error, please inform us immediately on telephone numbers (65) 531 2295 or (65) 531 2488, reverse charges if necessary.

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ANNEX

Re : PCT International Application No. PCT/SG98/00045
International Filing Date : June 19, 1998

Applicant refers to the March 10, 2000 Written Opinion issued by the International Preliminary Examining Authority in connection with the above-identified application. Applicant hereby submits the below response which applicant believes to fully overcome the rejections and objections raised.

Please replace pages 47-65 with the replacement pages attached hereto as Exhibit A.

The Examiner stated that IPEA is of the opinion that the present application lacks unity a priori since the subject-matter of present claims is not directed to one single invention as required by Rule 13.1-13.3 PCT but covers two separate inventions not linked by a common inventive concept, namely:

- invention 1: major surface antigen of hepatitis B virus having at amino acid residue 145 arginine instead of glycine (mutated form of the major surface antigen of hepatitis B virus) (claims 1-9, 10, 12-22, 24-30, 35, 37-47, 49-74 complete); and
- invention 2: major surface antigen of hepatitis B virus which does not necessarily have at amino acid residue 145 arginine in place of glycine but may have glycine instead of arginine at said position (wild-type form of the major surface antigen) (claims 11, 23, 31-34, 36, 48 complete)

In response, applicants respectfully traverse the International Preliminary Examination Authority's opinion that the subject application lacks unity of invention. Applicants contend that the claims relate to one invention or to a group of inventions so linked



as to form a common inventive concept and therefore, there is unity of invention. It appears that the Examiner has separated the claims into the following two groups: group 1 containing claims 1-9, 10, 12-22, 24-30, 35, 37-47, and 49-74; and group 2 containing claims 11, 23, 31-34, 36 and 48. Applicants respectfully point out that the mutation of amino acid residue number 145 from glycine to arginine of the major surface antigen corresponds to the mutation of amino acid residue 319 of Seq ID No. 3 from glycine to arginine of the large surface antigen. The description of Figure 5 in the specification teaches that amino acid residue number 319 of Seq ID No. 3 of the large surface antigen also contains the glycine to arginine mutation. Page 24 of the specification teaches that the large surface antigen overlaps the middle surface antigen and major surface antigen. Accordingly, the glycine to arginine mutation at residue 145 of the major surface antigen corresponds to a mutation of residue 319 of the large surface antigen. Accordingly, the invention of claims 11, 23, 31-34, 36 and 48 which recite "an amino acid sequence comprising residues 298-320 of SEQ ID No:3" forms a common inventive concept with the invention of claims 1-9, 10, 12-22, 24-30, 35, 37-47, and 49-74 since they both have the glycine to arginine mutation: the mutation of glycine to arginine at residue 145 of the major surface antigen corresponds to a mutation of residue 319 of the large surface antigen.

a) Novelty:

The Examiner stated that it is evident from the date of deposit defined in claim 1 and in the description that the strain according to claim 1 already was available before the priority date of the present application. The Examiner stated correspondingly, claim 1 lacks novelty (Art. 33(2) PCT). The Examiner stated that moreover, from the description of the present application (see page 2, second paragraph) it appears that the major surface antigen of hepatitis B virus having at amino acid residue 145 arginine in place of glycine also was already available before the filing (priority) date of present application. The Examiner stated therefore, novelty of



claims 21, 22, 24, 47, 73 and 74 cannot be acknowledged either. The Examiner stated with respect to claims 73 and 74 it is pointed out that an indication of use in a product claim is not considered suitable to limit the scope of such a claim (cf. Guidelines C-111 4.8 PCT).

In response, applicants respectfully traverse the Examiner's above rejection. With regard to claim 1, applicants deposited the virus with an International Depository Authority as established under the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. A copy of the deposit receipt is attached hereto as Exhibit B. The deposit did not make the virus available to the public before the priority date and therefore did not affect the novelty of the claimed invention. Accordingly, the claimed invention is novel.

With regard to claims 21, 22, 24, 47, 73 and 74, applicants without conceding the correctness of the Examiner's position but to expedite prosecution of the subject application have hereinabove amended claims 21, 22, 24, 47 and 73 to recite the deposit Accession No. Applicants contend that the claimed mutant virus was not available to the public prior to its priority date since it was deposited with International Depository Authority as established under the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. Applicants contend that this amendment obviates the above rejection and respectfully request that the Examiner reconsider and withdraw this ground of rejection.

The Examiner stated claims 11, 23, 31-33, 34, 36 and 48 cannot be considered to be novel since the sequence defined in these claims corresponds to the amino acid sequence of the wild-type major surface antigen of hepatitis B virus, the wild-type major surface antigen per se, antibodies directed against said wild-type protein and methods for preparing such antibodies.



Applicants respectfully contend that the invention of claims 11, 23, 31-33, 34, 36 and 48 is novel. As discussed above the mutation of amino acid residue number 145 from glycine to arginine of the major surface antigen corresponds to the mutation of amino acid residue 319 of Seq ID No. 3 from glycine to arginine of the large surface antigen. The description of Figure 5 in the specification teaches that amino acid residue number 319 of Seq ID No. 3 of the large surface antigen also contains the glycine to arginine mutation. Page 24 of the specification teaches that the large surface antigen overlaps the middle surface antigen and major surface antigen. Accordingly, the mutation of glycine to arginine at residue 145 of the major surface antigen corresponds to a mutation of residue 319 of the large surface antigen. Therefore, these claims do not embrace the wildtype protein since they all contain the glycine to arginine mutation.

b) Inventive step:

The Examiner stated the existence of the hepatitis B virus strain with the mutation at position 145 (arginine instead of glycine) was already known at the priority date of present application (see above). The Examiner stated that moreover, the association of said strain with the development of acute hepatitis B also is known in the art (see page 2, last paragraph of present application). The Examiner stated that thus, the provision of the mutated major surface antigen of said strain and/or screening methods appear to have been obvious to a person skilled in the art. The Examiner stated that therefore, present claims lack inventive activity and thus do not meet the requirements of Art. 33(3) PCT. -

In response, applicants contend that the claimed invention is inventive. As discussed above, the claimed viral strain was not available to the public before the priority date. Therefore, the sequence of this specific strain was not known until it was isolated and sequenced. Therefore, the strain with an arginine to glycine mutation was also not known and therefore is inventive.



Claims 45, 46, 65 and 66

The Examiner stated that the scope of claims 45, 46, 65, 66 is unclear since it is now known whether said claims are directed to a method of identifying a chemical compound or whether they are directed to the use of chemical compounds in a method for the manufacture of medicament.

In response, contend that the claims were sufficiently clear. Nevertheless, applicants without conceding the correctness of the Examiner's position but to expedite prosecution of the subject application have hereinabove amended the claims such that it is even more clear that they are directed to a method of identifying a chemical compound. Applicants contend that these amendments obviate the above rejection and respectfully request that the Examiner reconsider and withdraw this ground of rejection.

Claims 47-48

The Examiner stated that the term "derivative thereof" used in claims 47 and 48 renders the scope of said claims unclear (Art. 6 PCT) since it is not clear which peptides are covered by said expression and which are not.

In response, applicants without conceding the correctness of the Examiner's position but to expedite prosecution of the subject application have hereinabove amended claims 47-48 such that they no longer recite the words "derivative thereof." Applicants contend that this amendment obviates the above rejection and respectfully request that the Examiner reconsider and withdraw this ground of rejection.

Claims 52, 54 and 62

The Examiner stated the reference in claims 52, 54 and 62 should be checked (claims 45 and 46 are not directed to compositions but to methods and claim 61 does not relate to a method but to a use.)

In response, applicants without conceding the correctness of the Examiner's position but to expedite prosecution of the subject



application have hereinabove amended the references in claims 52, 54 and 62. Applicants contend that these amendments obviate the above rejection and respectfully request that the Examiner reconsider and withdraw this ground of rejection.



EXHIBIT A



What is claimed is:

1. An isolated strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-133 Oon Strain (Methionine to Threonine) and deposited under Accession Nos. P97121504, P97121505 and P97121506 with the European Collection of Cell Culture on 15th December 1997.
2. An isolated nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine rather than a methionine.
3. The isolated nucleic acid of claim 2, wherein the polypeptide is being encoded by nucleotides 155 through 835 of the nucleic acid sequence designated SEQ. I.D. No. 1.
4. The isolated nucleic acid of claim 3, comprising nucleotides "ACG" in position 551-553.
5. The isolated nucleic acid of claim 2, wherein the nucleic acid is DNA.
6. The isolated nucleic acid of claim 2, wherein the nucleic acid is RNA.
7. The isolated nucleic acid of claim 5, wherein the nucleic acid is cDNA.
8. The isolated nucleic acid of claim 5, wherein the nucleic acid is genomic DNA.



9. The isolated nucleic acid of claim 2, wherein the polypeptide has an amino acid sequence substantially identical to amino acid residues 174 through 400 of the amino acid sequence designated SEQ. I.D. No. 3.
10. An isolated nucleic acid which encodes a peptide, wherein the peptide is encoded by a nucleic acid molecule comprising nucleotides 527 through 595 of SEQ. I.D. No. 1.
11. An isolated nucleic acid which encodes a peptide, wherein the peptide has an amino acid sequence comprising amino acid residues 298 through 320 of the amino acid sequence designated SEQ. I.D. No. 3.
12. A vector comprising an isolated nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine rather than a methionine and operatively linked to a promoter of RNA transcription.
13. A vector comprising an isolated nucleic acid encoding a peptide, wherein the peptide is encoded by a nucleic acid molecule comprising nucleotides 527 through 595 of SEQ. I.D. No. 1.
14. The vector of claim 12 or 13, wherein the vector comprises viral DNA.
15. A host vector system for the production of a polypeptide which comprises the vector of claim 12 in a suitable host.
16. A host vector system for the production of a peptide which



comprises the vector of claim 13 in a suitable host.

17. A method of producing a polypeptide, which comprises growing the host vector system of claim 15 under suitable conditions permitting production of the polypeptide and recovering the polypeptide so produced.
18. A method of producing a peptide, which comprises growing the host vector system of claim 16 under suitable conditions permitting production of the polypeptide and recovering the polypeptide so produced.
19. A method of obtaining a polypeptide in purified form which comprises:
 - (a) introducing the vector of claim 12 into a suitable host cell;
 - (b) culturing the resulting host cell so as to produce the polypeptide;
 - (c) recovering the polypeptide produced into step (b); and
 - (d) purifying the polypeptide so recovered.
20. A method of obtaining a peptide in purified form which comprises:
 - (a) introducing the vector of claim 13 into a suitable host cell;
 - (b) culturing the resulting host cell so as to produce the polypeptide;
 - (c) recovering the polypeptide produced into step (b); and
 - (d) purifying the polypeptide so recovered.
21. A purified polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide



is a threonine rather than a methionine.

22. A purified polypeptide obtained from the method of claim 19.
23. A purified peptide, wherein the peptide has an amino acid sequence comprising amino acid residues 298 through 320 of the amino acid sequence designated SEQ. I.D. No.: 3.
24. A purified polypeptide obtained from the method of claim 20
25. An oligonucleotide of at least 15 nucleotides capable of specifically hybridizing with a unique sequence of nucleotides within a nucleic acid which encodes a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine rather than a methionine, without hybridizing to any sequence of nucleotides within a nucleic acid which encodes the major surface antigen of a wildtype hepatitis B virus.
26. The oligonucleotide of claim 25 comprising nucleotides 527 through 595 of SEQ. I.D. No. 1.
27. A method of obtaining antibodies to a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine, and not to the major surface antigen of a wildtype hepatitis B virus, comprising:
 - (a) obtaining the polypeptide in a purified form;
 - (b) immunizing an organism capable of producing antibodies



- against the purified polypeptide;
 - (c) collecting the produced antibodies;
 - (d) combining the produced antibodies and the purified polypeptide under conditions to form a complex; and
 - (e) determining which produced antibodies form a complex with the purified polypeptide so as to obtain antibodies to the polypeptide.
28. The method of claim 27, wherein the polypeptide is being encoded by nucleotides 155 through 335 of the nucleic acid sequence designated SEQ. I.D. No. 1.
29. The method of claim 27, wherein the polypeptide has an amino acid sequence substantially identical to amino acid residues 174 through 400 of the amino acid sequence designated SEQ. I.D. No. 3.
30. The method of claim 27, wherein the organism comprises a rabbit or a mouse.
31. A method of obtaining antibodies to a peptide, wherein the peptide has an amino acid sequence comprising amino acid residues 298 through 320 of the amino acid sequence designated SEQ. I.D. No. 3, comprising:
- (a) obtaining the peptide in a purified form;
 - (b) immunizing an organism capable of producing antibodies against the purified peptide;
 - (c) collecting the produced antibodies;
 - (d) combining the produced antibodies and the purified peptide under conditions to form a complex; and
 - (e) determining which produced antibodies form a complex with the purified peptide so as to obtain antibodies to the peptide.
32. The method of claim 31, wherein the organism comprises a



rabbit or a mouse.

33. The antibodies obtained in claim 27 or 31.
34. Monoclonal antibodies of the antibodies of claim 33.
35. Antibodies capable of detecting a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine, and incapable of detecting the major surface antigen of a wildtype hepatitis B virus.
36. Antibodies capable of detecting a peptide, wherein the peptide has an amino acid sequence comprising amino acid residues 298 through 320 of the amino acid sequence designated SEQ. I.D. No. 3.
37. Use of a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine for determining whether a subject is infected with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-133 Oon Strain (Methionine to Threonine), wherein such determination comprises:
 - (a) obtaining an appropriate nucleic acid sample from the subject; and
 - (b) determining whether the nucleic acid sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a



strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine.

38. The use of claim 37, wherein the nucleic acid sample in step (a) comprises mRNA corresponding to the transcript of DNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine, and wherein the determining of step (b) comprises:

- (i) contacting the mRNA with the oligonucleotide of claim 25 under conditions permitting binding of the mRNA to the oligonucleotide so as to form a complex;
- (ii) isolating the complex so formed; and
- (iii) identifying the mRNA in the isolated complex so as to thereby determine whether the mRNA is, or is derived from, a nucleic acid which encodes the polypeptide.

39. The use of claim 37, wherein the nucleic acid sample in step (a) comprises mRNA corresponding to the transcript of DNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine, and wherein the determining of step (b) comprises:



- (i) translating the mRNA under suitable conditions to obtain an amino acid sequence; and
 - (ii) comparing the amino acid sequence of step (i) with the amino acid sequence of the isolated nucleic acid of claim 9 so as to thereby determine whether the nucleic acid sample is, or is derived from, a nucleic acid which encodes the polypeptide.
40. The use of claim 37, wherein the determining of step (b) comprises:
- (i) amplifying the nucleic acid present in the sample of step (a); and
 - (ii) detecting the presence of polypeptide in the resulting amplified nucleic acid.
41. Use of an antibody that recognizes a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine for determining whether a subject is infected with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-133 Oon Strain (Methionine to Threonine), wherein such determination comprises:
- (a) obtaining an appropriate sample from the subject; and
 - (b) determining whether the sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine by contacting the sample under appropriate conditions to



bind to the antibodies of claim 35 or 36 so as to determine whether a subject is infected.

42. The use of claim 37, 38 or 41, wherein the isolated nucleic acid, oligonucleotide or antibody is labeled with a detectable marker.
43. The use the claim 42, wherein the detectable marker is a radioactive isotope, a fluorophor or an enzyme.
44. The use of claim 37, wherein the sample comprises blood, tissue or sera.
45. Use of a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine for determining whether a subject has a predisposition for hepatocellular carcinoma, which comprises:
 - (a) obtaining an appropriate nucleic acid sample from the subject; and
 - (b) determining whether the nucleic acid sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine.
46. The use of claim 45, wherein the nucleic acid sample in step (a) comprises mRNA corresponding to the transcript of DNA



encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine, and wherein the determining of step (b), which comprises:

- (i) contacting the mRNA with the oligonucleotide of claim 25 under conditions permitting binding of the mRNA to the oligonucleotide so as to form a complex;
- (ii) isolating the complex so formed; and
- (iii) identifying the mRNA in the isolated complex so as to thereby determine whether the mRNA is, or is derived from, a nucleic acid which encodes the polypeptide.

47. The use of claim 45, wherein the nucleic acid sample in step (a) comprises mRNA corresponding to the transcript of DNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine, and wherein the determining of step (b) comprises:

- (i) translating the mRNA under suitable conditions to obtain an amino acid sequence; and
- (ii) comparing the amino acid sequence of step (i) with the amino acid sequence of the isolated nucleic acid of claim 9 so as to thereby determine whether the nucleic acid sample is, or is derived from, a nucleic acid which encodes the polypeptide.

48. The use of claim 45, wherein the determining of step (b)



comprises:

- (i) amplifying the nucleic acid present in the sample of step (a); and
- (ii) detecting the presence of polypeptide in the resulting amplified nucleic acid.

49. Use of an antibody that recognizes a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine for determining whether the subject has a predisposition for hepatocellular carcinoma, wherein such determination comprises:
- (a) obtaining an appropriate sample from the subject; and
 - (b) determining whether the sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine by contacting the sample under appropriate conditions to bind to the antibodies of claim 35 or 36 so as to determine whether the subject has a predisposition for hepatocellular carcinoma.
50. The use of claim 46, 47 or 49, wherein the isolated nucleic acid, oligonucleotide or antibody is labeled with a detectable marker.



51. The use of claim 50, wherein the detectable marker is a radioactive isotope, a fluorophor or an enzyme.
52. The use of claim 45, wherein the sample comprises blood, tissue or sera.
53. A method for identifying a chemical compound for use in the manufacture of a medicament capable of treating infection by a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-133 Oon Strain (Methionine to Threonine) wherein the method for identifying the chemical compound comprises:
 - (a) contacting a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine, with the chemical compound under conditions permitting binding between the polypeptide and the chemical compound;
 - (b) detecting specific binding of the chemical compound to the polypeptide; and
 - (c) determining whether the chemical compound inhibits the polypeptide so as to identify a chemical compound which is capable of treating infection by the viral strain.
54. A method for identifying a chemical compound for use in the manufacture of a medicament capable of preventing infection by a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-133 Oon Strain (Methionine to Threonine), wherein the method for identifying the chemical compound comprises:
 - (a) contacting a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such



polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine, with the chemical compound under conditions permitting binding between the polypeptide and the chemical compound;

- (b) detecting specific binding of the chemical compound to the polypeptide; and
- (c) determining whether the chemical compound inhibits the polypeptide so as to identify a chemical compound which is capable of preventing infection by the viral strain.

55. A method for identifying a chemical compound for use in the manufacture of a medicament capable of treating hepatocellular carcinoma wherein the method for identifying the chemical compound comprises:

- (a) contacting a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine, with the chemical compound under conditions permitting binding between the polypeptide and the chemical compound;
- (b) detecting specific binding of the chemical compound to the polypeptide; and
- (c) determining whether the chemical compound inhibits the polypeptide so as to identify a chemical compound which is capable of treating hepatocellular carcinoma.

56. A method for identifying a chemical compound for use in the manufacture of a medicament capable of preventing hepatocellular carcinoma, wherein the method for identifying



the chemical compound comprises:

- (a) contacting a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine, with the chemical compound under conditions permitting binding between the polypeptide and the chemical compound;
 - (b) detecting specific binding of the chemical compound to the polypeptide; and
 - (c) determining whether the chemical compound inhibits the polypeptide so as to identify a chemical compound which is capable of preventing infection by the viral strain.
57. A composition comprising a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine, the amounts of such polypeptide being effective to stimulate or enhance antibody production in a subject, and a pharmaceutically acceptable.
58. A composition comprising a peptide, wherein the peptide has an amino acid sequence comprising amino acid residues 298 through 320 of the amino acid sequence designated SEQ. I.D. No. 3., the amounts of such peptide being effective to stimulate or enhance antibody production in a subject, and a pharmaceutically acceptable.
59. A composition comprising the chemical compound identified by the method of claim 55 in an amount effective to treat



hepatocellular carcinoma and a pharmaceutically effective carrier.

60. A composition comprising the chemical compound identified by the method of claim 56 in an amount effective to prevent hepatocellular carcinoma and a pharmaceutically effective carrier.
61. A composition comprising the chemical compound identified by the method of claim 53 in an amount effective to treat a strain of hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-133 Oon Strain (Methionine to Threonine) and a pharmaceutically effective carrier.
62. A composition comprising the chemical compound identified by the method of claim 54 in an amount effective to prevent infection by a strain of hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-133 Oon Strain (Methionine to Threonine) and a pharmaceutically effective carrier.
63. Use of the composition of claim 57 or 58 for treating a subject infected with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-133 Oon Strain (Methionine to Threonine).
64. Use of the composition of claim 61 treating a subject infected with a strain of hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-133 Oon Strain (Methionine to Threonine).
65. Use of the composition of claim 57 or 58 for preventing infection by a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-133 Oon Strain (Methionine to Threonine) in a subject.



66. Use of the composition of claim 62 for preventing infection by a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-133 Oon Strain (Methionine to Threonine) in a subject.
67. Use of the composition of claim 57 or 58 for treating a subject with hepatocellular carcinoma.
68. Use of the composition of claim 59 for treating a subject with hepatocellular carcinoma.
69. Use of the composition of claim 57 or 59 for preventing hepatocellular carcinoma in a subject.
70. Use of the composition of claim 60 for preventing hepatocellular carcinoma in a subject.
71. A method of screening bodily fluids from a subject for a strain of hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-133 Oon Strain (Methionine to Threonine) which comprises:
 - (a) obtaining an appropriate sample of bodily fluid from the subject;
 - (b) determining the presence of a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine in the sample of step (a) so as to screen the sample for the strain.
72. The method of claim 71, wherein the bodily fluid comprises blood, sera, or a nucleic acid sample of blood or sera.



73. A hepatitis vaccine, comprising a mutant form of the surface antigen of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the wildtype amino acid sequence of the major surface antigen of hepatitis B in that the amino acid at position number 133 of such polypeptide is a threonine rather than a methionine.
74. The vaccine of claim 73, further comprising an adjuvant.



EXHIBIT B





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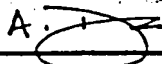




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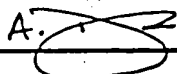




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**IMMEDIATE
ATTENTION**

Dear Sir,

**Demand under Article 31 of the Patent Cooperation Treaty
PCT International Application No. PCT/SG98/00045
Entitled: A Vaccine-Induced Hepatitis B Viral Strain and Uses Thereof
Applicant: Government of the Republic of Singapore**

Thank you for the Written Opinion dated 10 March 2000 issued in connection with the International Preliminary Examination Report.

Our client wishes to file arguments and amendments in response to the Written Opinion dated 10 March 2000. In accordance with Rule 66.3, we submit herewith the following:-

- (i) A copy of replacement sheets (pages 35-51), and
- (ii) An annex stating the applicant's arguments on response to the Written Opinion.

We look forward to receiving a favourable Examination Report as soon as possible.

Yours faithfully,

Morris John
Drew & Napier
Engl.

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MP: Multi Polling
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ATTORNEY-GENERAL'S CHAMBERS,
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SINGAPORE 179803

In reply, please quote our reference number:

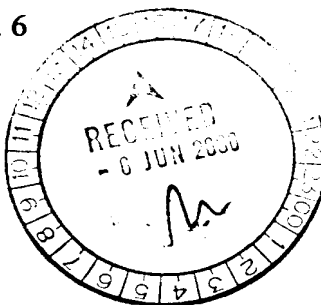
AG/CIV/GM20/MOH/1997/17 Vol. 6
CG/PHM/PAT/8013374

6 June 2000

M/s Drew & Napier
20 Raffles Place #17-00
Ocean Towers
Singapore 048620

Attn: Ms Cecelia Girvin

Dear Ms Girvin



DID 3325909
FAX 3325984

**IMMEDIATE
BY HAND**

PCT International Application No. PCT/SG98/00045
Applicant: Government of Republic of Singapore
Title: A Vaccine-Induced Hepatitis B Viral Strain & Uses Thereof

We refer to the above matter.

2 Enclosed, please find the draft response that our US patent attorneys assisted us in preparing. With regard to the Certificate of Deposit of micro-organism, copies of the three Certificates are attached as well. The three Certificates are to be attached as **Exhibit B** to the response. We trust that the draft is in order and that you will be able to respond to the Written Opinion of the European Patent Office before the deadline of **10 June 2000**.

Yours faithfully

PHUA WEE CHUAN
for ATTORNEY-GENERAL
SINGAPORE

Virus Pat2



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June 5, 2000

VIA FACSIMILE

Mr. Phua Wee Chuan
Attorney-General Singapore
1 Coleman Street #10-00
Singapore 179803

Re: PCT International Application No. PCT/SG98/00045, filed June 19, 1998 on behalf of The Government of the Republic of Singapore, et al., for A Vaccine-Induced Hepatitis B Viral Strain and Uses Thereof - Your Ref. AG/CIV/GM20/MOH/1997/17 Vol. 10; Our Docket 54620-PCT

Dear Mr. Phua:

Thank you for your April 20 and May 12, 2000 letters. In response, I enclose a draft Amendment in Response to March 10, 2000 Written Opinion issued by the European Patent Office in connection with the above-identified application. We trust that this draft will enable you to prepare and file a response to the March 10, 2000 Written Opinion prior to the June 10, 2000 deadline for doing so.

We have enclosed the revised claims as Exhibit A. I have also enclosed a claims worksheet for your convenience.

Please note that we do not have a complete prosecution file for the subject application. The enclosed response is based on the premise that the virus was deposited with an International Depository Authority as established under the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure such that the virus was not available to the public prior to the priority date.

Please attach a copy of the deposit receipt as Exhibit B of the response.

In addition, we note that the priority date for the subject application is June 19, 1998. The deadline for entering the national or regional stage, whichever was appropriate, for the subject application under Chapter I of the Patent Cooperation treaty was twenty months (20) or twenty one (21) months from the priority date, i.e. February 19, 2000 or March 19, 2000.



Mr. Phua Wee Chuan
June 5, 2000
Page 2

If you did not enter the national or regional stage under Chapter I and instead filed a Demand for an International Preliminary Examination under Chapter II of the Patent Cooperation Treaty by 19 months from the priority date, i.e. January 19, 2000, the deadline for entering the national or regional stage, whichever is appropriate, in the elected countries is thirty (30) or thirty-one (31) months from the priority date, i.e. December 19, 2000 or January 19, 2001, depending on the specific country or region in question.

Please advise us whether the subject application is proceeding under Chapter I or Chapter II.

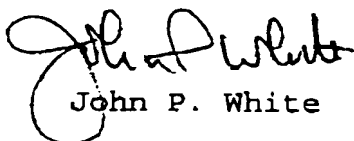
Please note that there is no United States patent application pending. The deadline for entering the United States under Chapter II would be December 19, 2000. Please advise us whether you wish us to enter the subject application into the United States by this deadline. We will take no action with respect to the entering the subject application in the United States unless we receive written instructions from you to do so.

Please note that failure to enter the national or regional stage in these countries or regions by the applicable deadlines will result in the abandonment of all patent rights in these countries or regions otherwise available based on the subject PCT Application.

Please acknowledge receipt of this facsimile and the enclosures by return facsimile.

If you have any questions or comments, please contact us.

Sincerely,


John P. White

JPW/SHS:cf
Enclosures

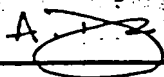




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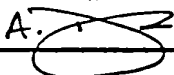




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Attention: I.Scheffzyk

Sir:

Re: International Application No. PCT/SG98/00045

International Filing Date: June 19, 1998

Applicant refers to the March 10, 2000 Written Opinion issued by the International Preliminary Examining Authority in connection with the above-identified application. Applicant hereby submits the below response which applicant believes to fully overcome the rejections and objections raised.

Please replace pages 47-65 with the replacement pages attached hereto as Exhibit A.

The Examiner stated that the IPEA is of the opinion that the present application lacks unity a priori since the subject-matter of present claims is not directed to one single invention as required by Rule 13.1-13.3 PCT but covers two separate inventions not linked by a common inventive concept, namely: -

invention 1: major surface antigen of hepatitis B virus having at amino acid residue 145 arginine instead of glycine (mutated form of the major surface antigen of hepatitis B virus) (claims 1-9, 10, 12-22, 24-30, 35, 37-47, 49-74)



Applicants: The Government of the Republic of Singapore
 Re: International Application No. PCT/SG98/00046
 Filed: June 19, 1998
 Page 2

complete); and

invention 2: major surface antigen of hepatitis B virus which does not necessarily have at amino acid residue 145 arginine in place of glycine but may have glycine instead of arginine at said position (wild-type form of the major surface antigen) (claims 11, 23, 31-34, 36, 48 complete)

In response, applicants respectfully traverse the International Preliminary Examination Authority's opinion that the subject application lacks unity of invention. Applicants contend that the claims relate to one invention or to a group of inventions so linked as to form a common inventive concept and therefore, there is unity of invention. It appears that the Examiner has separated the claims into the following two groups: group 1 containing claims 1-9, 10, 12-22, 24-30, 35, 37-47, and 49-74; and group 2 containing claims 11, 23, 31-34, 36 and 48. Applicants respectfully point out that the mutation of amino acid residue number 145 from glycine to arginine of the major surface antigen corresponds to the mutation of amino acid residue 319 of Seq ID No. 3 from glycine to arginine of the large surface antigen. The description of Figure 5 in the specification teaches that amino acid residue number 319 of Seq ID No. 3 of the large surface antigen also contains the glycine to arginine mutation. Page 24 of the specification teaches that the large surface antigen overlaps the middle surface antigen and major surface antigen. Accordingly, the glycine to arginine mutation at residue 145 of the major surface antigen corresponds to a mutation of residue 319 of the large surface antigen. Accordingly, the invention of claims 11, 23, 31-34, 36 and 48 which recite "an amino acid sequence comprising residues 298-320 of SEQ ID NO:3" forms a common inventive concept with the invention of claims 1-9, 10, 12-



Applicants: The Government of the Republic of Singapore
Re: International Application No. PCT/SG98/00046
Filed: June 19, 1998
Page 3

22, 24-30, 35, 37-47, and 49-74 since they both have the glycine to arginine mutation: the mutation of glycine to arginine at residue 145 of the major surface antigen corresponds to a mutation of residue 319 of the large surface antigen.

a) Novelty:

The Examiner stated that it is evident from the date of deposit defined in claim 1 and in the description that the strain according to claim 1 already was available before the priority date of the present application. The Examiner stated correspondingly, claim 1 lacks novelty (Art. 33(2) PCT). The Examiner stated that moreover, from the description of the present application (see page 2, second paragraph) it appears that the major surface antigen of hepatitis B virus having at amino acid residue 145 arginine in place of glycine also was already available before the filing (priority) date of present application. The Examiner stated therefore, novelty of claims 21, 22, 24, 47, 73 and 74 cannot be acknowledged either. The Examiner stated with respect to claims 73 and 74 it is pointed out that an indication of use in a product claim is not considered suitable to limit the scope of such a claim (cf. Guidelines C-111 4.8 PCT).

In response, applicants respectfully traverse the Examiner's above rejection. With regard to claim 1, applicants deposited the virus with an International Depository Authority as established under the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. A copy of the deposit receipt is attached hereto as Exhibit B. The deposit did not make the virus available to the public before the priority date and therefore did not affect the novelty of the claimed invention. Accordingly, the claimed invention is novel.



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With regard to claims 21,22,24,47,73 and 74, applicants without conceding the correctness of the Examiner's position but to expedite prosecution of the subject application have hereinabove amended claims 21, 22, 24, 47 and 73 to recite the deposit Accession No. Applicants contend that the claimed mutant virus was not available to the public prior to its priority date since it was deposited with International Depository Authority as established under the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. Applicants contend that this amendment obviates the above rejection and respectfully request that the Examiner reconsider and withdraw this ground of rejection.

The Examiner stated claims 11, 23, 31-33, 34, 36 and 48 cannot be considered to be novel since the sequence defined in these claims corresponds to the amino acid sequence of the wild-type major surface antigen of hepatitis B virus, the wild-type major surface antigen per se, antibodies directed against said wild-type protein and methods for preparing such antibodies.

Applicants respectfully contend that the invention of claims 11, 23, 31-33, 34, 36 and 48 is novel. As discussed above the mutation of amino acid residue number 145 from glycine to arginine of the major surface antigen corresponds to the mutation of amino acid residue 319 of Seq ID No. 3 from glycine to arginine of the large surface antigen. The description of Figure 5 in the specification teaches that amino acid residue number 319 of Seq ID No. 3 of the large surface antigen also contains the glycine to arginine mutation. Page 24 of the specification teaches that the large surface antigen overlaps the middle surface antigen and major surface antigen. Accordingly, the mutation of glycine to arginine at residue 145 of the major surface antigen corresponds to a mutation of residue 319 of the large surface antigen. Therefore,



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Examiner's position but to expedite prosecution of the subject application have hereinabove amended the claims such that it is even more clear that they are directed to a method of identifying a chemical compound. Applicants contend that these amendments obviate the above rejection and respectfully request that the Examiner reconsider and withdraw this ground of rejection.

Claims 47-48

The Examiner stated that the term "derivative thereof" used in claims 47 and 48 renders the scope of said claims unclear (Art. 6 PCT) since it is not clear which peptides are covered by said expression and which are not.

In response, applicants without conceding the correctness of the Examiner's position but to expedite prosecution of the subject application have hereinabove amended claims 47-48 such that they no longer recite the words "derivative thereof." Applicants contend that this amendment obviates the above rejection and respectfully request that the Examiner reconsider and withdraw this ground of rejection.

Claims 52, 54 and 62

The Examiner stated the reference in claims 52, 54 and 62 should be checked (claims 45 and 46 are not directed to compositions but to methods and claim 61 does not relate to a method but to a use.)

In response, applicants without conceding the correctness of the Examiner's position but to expedite prosecution of the subject application have hereinabove amended the references in claims 52, 54 and 62. Applicants contend that these amendments obviate the above rejection and respectfully request that the Examiner reconsider and withdraw this ground of rejection.



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these claims do not embrace the wildtype protein since they all contain the glycine to arginine mutation.

b) Inventive step:

The Examiner stated the existence of the hepatitis B virus strain with the mutation at position 145 (arginine instead of glycine) was already known at the priority date of present application (see above). The Examiner stated that moreover, the association of said strain with the development of acute hepatitis B also is known in the art (see page 2, last paragraph of present application). The Examiner stated that thus, the provision of the mutated major surface antigen of said strain and/or screening methods appear to have been obvious to a person skilled in the art. The Examiner stated that therefore, present claims lack inventive activity and thus do not meet the requirements of Art. 33(3) PCT.

In response, applicants contend that the claimed invention is inventive. As discussed above, the claimed viral strain was not available to the public before the priority date. Therefore, the sequence of this specific strain was not known until it was isolated and sequenced. Therefore, the strain with an arginine to glycine mutation was also not known and therefore is inventive.

Claims 45, 46, 65 and 66

The Examiner stated that the scope of claims 45, 46, 65, 66 is unclear since it is not known whether said claims are directed to a method of identifying a chemical compound or whether they are directed to the use of chemical compounds in a method for the manufacture of a medicament.

In response, applicants contend that the claims were sufficiently clear. Nevertheless, applicants without conceding the correctness of the



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Respectfully submitted

[name]



EXHIBIT A



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What is claimed is:

1. An isolated strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-133 Oon Strain (Methionine to Threonine) and deposited under Accession No. _____.
2. An isolated nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine rather than a methionine.
3. The isolated nucleic acid of claim 2, wherein the polypeptide is being encoded by nucleotides 155 through 835 of the nucleic acid sequence designated SEQ. I.D. No. 1.
4. The isolated nucleic acid of claim 3, comprising nucleotides "ACG" in position 551-553.
5. The isolated nucleic acid of claim 2, wherein the nucleic acid is DNA.
6. The isolated nucleic acid of claim 2, wherein the nucleic acid is RNA.
7. The isolated nucleic acid of claim 5, wherein the nucleic acid is cDNA.
8. The isolated nucleic acid of claim 5, wherein the nucleic acid is genomic DNA.



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9. The isolated nucleic acid of claim 2, wherein the polypeptide has an amino acid sequence substantially identical to amino acid residues 174 through 400 of the amino acid sequence designated SEQ. I.D. No. 3.
10. An isolated nucleic acid which encodes a peptide, wherein the peptide is encoded by a nucleic acid molecule comprising nucleotides 527 through 595 of SEQ. I.D. No. 1.
11. An isolated nucleic acid which encodes a peptide, wherein the peptide has an amino acid sequence comprising amino acid residues 298 through 320 of the amino acid sequence designated SEQ. I.D. No. 3.
12. A vector comprising an isolated nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine rather than a methionine and operatively linked to a promoter of RNA transcription.
13. A vector comprising an isolated nucleic acid encoding a peptide, wherein the peptide is encoded by a nucleic acid molecule comprising nucleotides 527 through 595 of SEQ. I.D. No. 1.
14. The vector of claim 12 or 13, wherein the vector comprises viral DNA.
15. A host vector system for the production of a polypeptide which comprises the vector of claim 12 in a suitable host.
16. A host vector system for the production of a peptide which



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comprises the vector of claim 13 in a suitable host.

17. A method of producing a polypeptide, which comprises growing the host vector system of claim 15 under suitable conditions permitting production of the polypeptide and recovering the polypeptide so produced.
18. A method of producing a peptide, which comprises growing the host vector system of claim 16 under suitable conditions permitting production of the polypeptide and recovering the polypeptide so produced.
19. A method of obtaining a polypeptide in purified form which comprises:
 - (a) introducing the vector of claim 12 into a suitable host cell;
 - (b) culturing the resulting host cell so as to produce the polypeptide;
 - (c) recovering the polypeptide produced into step (b); and
 - (d) purifying the polypeptide so recovered.
20. A method of obtaining a peptide in purified form which comprises:
 - (a) introducing the vector of claim 13 into a suitable host cell;
 - (b) culturing the resulting host cell so as to produce the polypeptide;
 - (c) recovering the polypeptide produced into step (b); and
 - (d) purifying the polypeptide so recovered.
21. A purified polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide



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is a threonine rather than a methionine.

22. A purified polypeptide obtained from the method of claim 19.
23. A purified peptide, wherein the peptide has an amino acid sequence comprising amino acid residues 298 through 320 of the amino acid sequence designated SEQ. I.D. No.: 3.
24. A purified polypeptide obtained from the method of claim 20
25. An oligonucleotide of at least 15 nucleotides capable of specifically hybridizing with a unique sequence of nucleotides within a nucleic acid which encodes a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine rather than a methionine, without hybridizing to any sequence of nucleotides within a nucleic acid which encodes the major surface antigen of a wildtype hepatitis B virus.
26. The oligonucleotide of claim 25 comprising nucleotides 527 through 595 of SEQ. I.D. No. 1.
27. A method of obtaining antibodies to a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine, and not to the major surface antigen of a wildtype hepatitis B virus, comprising:
 - (a) obtaining the polypeptide in a purified form;
 - (b) immunizing an organism capable of producing antibodies



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- against the purified polypeptide;
 - (c) collecting the produced antibodies;
 - (d) combining the produced antibodies and the purified polypeptide under conditions to form a complex; and
 - (e) determining which produced antibodies form a complex with the purified polypeptide so as to obtain antibodies to the polypeptide.
28. The method of claim 27, wherein the polypeptide is being encoded by nucleotides 155 through 835 of the nucleic acid sequence designated SEQ. I.D. No. 1.
29. The method of claim 27, wherein the polypeptide has an amino acid sequence substantially identical to amino acid residues 174 through 400 of the amino acid sequence designated SEQ. I.D. No. 3.
30. The method of claim 27, wherein the organism comprises a rabbit or a mouse.
31. A method of obtaining antibodies to a peptide, wherein the peptide has an amino acid sequence comprising amino acid residues 298 through 320 of the amino acid sequence designated SEQ. I.D. No. 3, comprising:
- (a) obtaining the peptide in a purified form;
 - (b) immunizing an organism capable of producing antibodies against the purified peptide;
 - (c) collecting the produced antibodies;
 - (d) combining the produced antibodies and the purified peptide under conditions to form a complex; and
 - (e) determining which produced antibodies form a complex with the purified peptide so as to obtain antibodies to the peptide.
32. The method of claim 31, wherein the organism comprises a



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rabbit or a mouse.

33. The antibodies obtained in claim 27 or 31.
34. Monoclonal antibodies of the antibodies of claim 33.
35. Antibodies capable of detecting a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine, and incapable of detecting the major surface antigen of a wildtype hepatitis B virus.
36. Antibodies capable of detecting a peptide, wherein the peptide has an amino acid sequence comprising amino acid residues 298 through 320 of the amino acid sequence designated SEQ. I.D. No. 3.
37. Use of a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine for determining whether a subject is infected with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-133 Oon Strain (Methionine to Threonine), wherein such determination comprises:
 - (a) obtaining an appropriate nucleic acid sample from the subject; and
 - (b) determining whether the nucleic acid sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a



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strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine.

38. The use of claim 37, wherein the nucleic acid sample in step (a) comprises mRNA corresponding to the transcript of DNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine, and wherein the determining of step (b) comprises:
 - (i) contacting the mRNA with the oligonucleotide of claim 25 under conditions permitting binding of the mRNA to the oligonucleotide so as to form a complex;
 - (ii) isolating the complex so formed; and
 - (iii) identifying the mRNA in the isolated complex so as to thereby determine whether the mRNA is, or is derived from, a nucleic acid which encodes the polypeptide.
39. The use of claim 37, wherein the nucleic acid sample in step (a) comprises mRNA corresponding to the transcript of DNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine, and wherein the determining of step (b) comprises:



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- (i) translating the mRNA under suitable conditions to obtain an amino acid sequence; and
- (ii) comparing the amino acid sequence of step (i) with the amino acid sequence of the isolated nucleic acid of claim 9 so as to thereby determine whether the nucleic acid sample is, or is derived from, a nucleic acid which encodes the polypeptide.

40. The use of claim 37, wherein the determining of step (b) comprises:

- (i) amplifying the nucleic acid present in the sample of step (a); and
- (ii) detecting the presence of polypeptide in the resulting amplified nucleic acid.

41. Use of an antibody that recognizes a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine for determining whether a subject is infected with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-133 Oon Strain (Methionine to Threonine), wherein such determination comprises:

- (a) obtaining an appropriate sample from the subject; and
- (b) determining whether the sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine by contacting the sample under appropriate conditions to



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bind to the antibodies of claim 35 or 36 so as to determine whether a subject is infected.

42. The use of claim 37, 38 or 41, wherein the isolated nucleic acid, oligonucleotide or antibody is labeled with a detectable marker.
43. The use the claim 42, wherein the detectable marker is a radioactive isotope, a fluorophor or an enzyme.
44. The use of claim 37, wherein the sample comprises blood, tissue or sera.
45. Use of a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine for determining whether a subject has a predisposition for hepatocellular carcinoma, which comprises:
 - (a) obtaining an appropriate nucleic acid sample from the subject; and
 - (b) determining whether the nucleic acid sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine.
46. The use of claim 45, wherein the nucleic acid sample in step (a) comprises mRNA corresponding to the transcript of DNA



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encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine, and wherein the determining of step (b), which comprises:

- (i) contacting the mRNA with the oligonucleotide of claim 25 under conditions permitting binding of the mRNA to the oligonucleotide so as to form a complex;
- (ii) isolating the complex so formed; and
- (iii) identifying the mRNA in the isolated complex so as to thereby determine whether the mRNA is, or is derived from, a nucleic acid which encodes the polypeptide.

47. The use of claim 45, wherein the nucleic acid sample in step (a) comprises mRNA corresponding to the transcript of DNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine, and wherein the determining of step (b) comprises:

- (i) translating the mRNA under suitable conditions to obtain an amino acid sequence; and
- (ii) comparing the amino acid sequence of step (i) with the amino acid sequence of the isolated nucleic acid of claim 9 so as to thereby determine whether the nucleic acid sample is, or is derived from, a nucleic acid which encodes the polypeptide.

48. The use of claim 45, wherein the determining of step (b)



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comprises:

- (i) amplifying the nucleic acid present in the sample of step (a); and
- (ii) detecting the presence of polypeptide in the resulting amplified nucleic acid.

49. Use of an antibody that recognizes a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine for determining whether the subject has a predisposition for hepatocellular carcinoma, wherein such determination comprises:

- (a) obtaining an appropriate sample from the subject; and
- (b) determining whether the sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine by contacting the sample under appropriate conditions to bind to the antibodies of claim 35 or 36 so as to determine whether the subject subject has a predisposition for hepatocellular carcinoma.

50. The use of claim 46, 47 or 49, wherein the isolated nucleic acid, oligonucleotide or antibody is labeled with a detectable marker.



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51. The use of claim 50, wherein the detectable marker is a radioactive isotope, a fluorophor or an enzyme.
52. The use of claim 45, wherein the sample comprises blood, tissue or sera.
53. A method for identifying a chemical compound for use in the manufacture of a medicament capable of treating infection by a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-133 Oon Strain (Methionine to Threonine) wherein the method for identifying the chemical compound comprises:
 - (a) contacting a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine, with the chemical compound under conditions permitting binding between the polypeptide and the chemical compound;
 - (b) detecting specific binding of the chemical compound to the polypeptide; and
 - (c) determining whether the chemical compound inhibits the polypeptide so as to identify a chemical compound which is capable of treating infection by the viral strain.
54. A method for identifying a chemical compound for use in the manufacture of a medicament capable of preventing infection by a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-133 Oon Strain (Methionine to Threonine), wherein the method for identifying the chemical compound comprises:
 - (a) contacting a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such



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polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine, with the chemical compound under conditions permitting binding between the polypeptide and the chemical compound;

- (b) detecting specific binding of the chemical compound to the polypeptide; and
 - (c) determining whether the chemical compound inhibits the polypeptide so as to identify a chemical compound which is capable of preventing infection by the viral strain.
55. A method for identifying a chemical compound for use in the manufacture of a medicament capable of treating hepatocellular carcinoma wherein the method for identifying the chemical compound comprises:
- (a) contacting a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine, with the chemical compound under conditions permitting binding between the polypeptide and the chemical compound;
 - (b) detecting specific binding of the chemical compound to the polypeptide; and
 - (c) determining whether the chemical compound inhibits the polypeptide so as to identify a chemical compound which is capable of treating hepatocellular carcinoma.
56. A method for identifying a chemical compound for use in the manufacture of a medicament capable of preventing hepatocellular carcinoma, wherein the method for identifying



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the chemical compound comprises:

- (a) contacting a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine, with the chemical compound under conditions permitting binding between the polypeptide and the chemical compound;
 - (b) detecting specific binding of the chemical compound to the polypeptide; and
 - (c) determining whether the chemical compound inhibits the polypeptide so as to identify a chemical compound which is capable of preventing infection by the viral strain.
57. A composition comprising a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine, the amounts of such polypeptide being effective to stimulate or enhance antibody production in a subject, and a pharmaceutically acceptable.
58. A composition comprising a peptide, wherein the peptide has an amino acid sequence comprising amino acid residues 298 through 320 of the amino acid sequence designated SEQ. I.D. No. 3., the amounts of such peptide being effective to stimulate or enhance antibody production in a subject, and a pharmaceutically acceptable.
59. A composition comprising the chemical compound identified by the method of claim 55 in an amount effective to treat



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hepatocellular carcinoma and a pharmaceutically effective carrier.

60. A composition comprising the chemical compound identified by the method of claim 56 in an amount effective to prevent hepatocellular carcinoma and a pharmaceutically effective carrier.
61. A composition comprising the chemical compound identified by the method of claim 53 in an amount effective to treat a strain of hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-133 Oon Strain (Methionine to Threonine) and a pharmaceutically effective carrier.
62. A composition comprising the chemical compound identified by the method of claim 54 in an amount effective to prevent infection by a strain of hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-133 Oon Strain (Methionine to Threonine) and a pharmaceutically effective carrier.
63. Use of the composition of claim 57 or 58 for treating a subject infected with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-133 Oon Strain (Methionine to Threonine).
64. Use of the composition of claim 61 treating a subject infected with a strain of hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-133 Oon Strain (Methionine to Threonine).
65. Use of the composition of claim 57 or 58 for preventing infection by a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-133 Oon Strain (Methionine to Threonine) in a subject.



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66. Use of the composition of claim 62 for preventing infection by a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-133 Oon Strain (Methionine to Threonine) in a subject.
67. Use of the composition of claim 57 or 58 for treating a subject with hepatocellular carcinoma.
68. Use of the composition of claim 59 for treating a subject with hepatocellular carcinoma.
69. Use of the composition of claim 57 or 59 for preventing hepatocellular carcinoma in a subject.
70. Use of the composition of claim 60 for preventing hepatocellular carcinoma in a subject.
71. A method of screening bodily fluids from a subject for a strain of hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-133 Oon Strain (Methionine to Threonine) which comprises:
 - (a) obtaining an appropriate sample of bodily fluid from the subject;
 - (b) determining the presence of a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine in the sample of step (a) so as to screen the sample for the strain.
72. The method of claim 71, wherein the bodily fluid comprises blood, sera, or a nucleic acid sample of blood or sera.



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73. A hepatitis vaccine, comprising a mutant form of the surface antigen of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the wildtype amino acid sequence of the major surface antigen of hepatitis B in that the amino acid at position number 133 of such polypeptide is a threonine rather than a methionine.
74. The vaccine of claim 73, further comprising an adjuvant.



CLAIMS WORKSHEET



What is claimed is:

1. An isolated strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-133 Oon Strain (Methionine to Threonine) and deposited under Accession No. _____.
2. An isolated nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine rather than a methionine.
3. The isolated nucleic acid of claim 2, wherein the polypeptide is being encoded by nucleotides 155 through 835 of the nucleic acid sequence designated SEQ. I.D. No. 1.
4. The isolated nucleic acid of claim 3, comprising nucleotides "ACG" in position 551-553.
5. The isolated nucleic acid of claim 2, wherein the nucleic acid is DNA.
6. The isolated nucleic acid of claim 2, wherein the nucleic acid is RNA.
7. The isolated nucleic acid of claim 5, wherein the nucleic acid is cDNA.
8. The isolated nucleic acid of claim 5, wherein the nucleic acid is genomic DNA.
9. The isolated nucleic acid of claim 2, wherein the polypeptide



has an amino acid sequence substantially identical to amino acid residues 174 through 400 of the amino acid sequence designated SEQ. I.D. No. 3.

10. An isolated nucleic acid which encodes a peptide, wherein the peptide is encoded by a nucleic acid molecule comprising nucleotides 527 through 595 of SEQ. I.D. No. 1.
11. An isolated nucleic acid which encodes a peptide, wherein the peptide has an amino acid sequence comprising amino acid residues 298 through 320 of the amino acid sequence designated SEQ. I.D. No. 3.
12. A vector comprising an isolated nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine rather than a methionine and operatively linked to a promoter of RNA transcription.
13. A vector comprising an isolated nucleic acid encoding a peptide, wherein the peptide is encoded by a nucleic acid molecule comprising nucleotides 527 through 595 of SEQ. I.D. No. 1.
14. The vector of claim 12 or 13, wherein the vector comprises viral DNA.
15. A host vector system for the production of a polypeptide which comprises the vector of claim 12 in a suitable host.
16. A host vector system for the production of a peptide which comprises the vector of claim 13 in a suitable host.



17. A method of producing a polypeptide, which comprises growing the host vector system of claim 15 under suitable conditions permitting production of the polypeptide and recovering the polypeptide so produced.
18. A method of producing a peptide, which comprises growing the host vector system of claim 16 under suitable conditions permitting production of the polypeptide and recovering the polypeptide so produced.
19. A method of obtaining a polypeptide in purified form which comprises:
 - (a) introducing the vector of claim 12 into a suitable host cell;
 - (b) culturing the resulting host cell so as to produce the polypeptide;
 - (c) recovering the polypeptide produced into step (b); and
 - (d) purifying the polypeptide so recovered.
20. A method of obtaining a peptide in purified form which comprises:
 - (a) introducing the vector of claim 13 into a suitable host cell;
 - (b) culturing the resulting host cell so as to produce the polypeptide;
 - (c) recovering the polypeptide produced into step (b); and
 - (d) purifying the polypeptide so recovered.
21. A purified polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine rather than a methionine.
22. A purified polypeptide obtained from the method of claim 19.



23. A purified peptide, wherein the peptide has an amino acid sequence comprising amino acid residues 298 through 320 of the amino acid sequence designated SEQ. I.D. No.: 3.
24. A purified polypeptide obtained from the method of claim 20
25. An oligonucleotide of at least 15 nucleotides capable of specifically hybridizing with a unique sequence of nucleotides within a nucleic acid which encodes a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine rather than a methionine, without hybridizing to any sequence of nucleotides within a nucleic acid which encodes the major surface antigen of a wildtype hepatitis B virus.
26. The oligonucleotide of claim 25 comprising nucleotides 527 through 595 of SEQ. I.D. No. 1.
27. A method of obtaining antibodies to a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine, and not to the major surface antigen of a wildtype hepatitis B virus, comprising:
 - (a) obtaining the polypeptide in a purified form;
 - (b) immunizing an organism capable of producing antibodies against the purified polypeptide;
 - (c) collecting the produced antibodies;
 - (d) combining the produced antibodies and the purified polypeptide under conditions to form a complex; and
 - (e) determining which produced antibodies form a complex with



the purified polypeptide so as to obtain antibodies to the polypeptide.

28. The method of claim 27, wherein the polypeptide is being encoded by nucleotides 155 through 835 of the nucleic acid sequence designated SEQ. I.D. No. 1.
29. The method of claim 27, wherein the polypeptide has an amino acid sequence substantially identical to amino acid residues 174 through 400 of the amino acid sequence designated SEQ. I.D. No. 3.
30. The method of claim 27, wherein the organism comprises a rabbit or a mouse.
31. A method of obtaining antibodies to a peptide, wherein the peptide has an amino acid sequence comprising amino acid residues 298 through 320 of the amino acid sequence designated SEQ. I.D. No. 3, comprising:
 - (a) obtaining the peptide in a purified form;
 - (b) immunizing an organism capable of producing antibodies against the purified peptide;
 - (c) collecting the produced antibodies;
 - (d) combining the produced antibodies and the purified peptide under conditions to form a complex; and
 - (e) determining which produced antibodies form a complex with the purified peptide so as to obtain antibodies to the peptide.
32. The method of claim 31, wherein the organism comprises a rabbit or a mouse.
33. The antibodies obtained in claim 27 or 31.
34. Monoclonal antibodies of the antibodies of claim 33.



35. Antibodies capable of detecting a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine, and incapable of detecting the major surface antigen of a wildtype hepatitis B virus.
36. Antibodies capable of detecting a peptide, wherein the peptide has an amino acid sequence comprising amino acid residues 298 through 320 of the amino acid sequence designated SEQ. I.D. No. 3.
37. Use of a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine for determining whether a subject is infected with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-133 Oon Strain (Methionine to Threonine), wherein such determination comprises:
 - (a) obtaining an appropriate nucleic acid sample from the subject; and
 - (b) determining whether the nucleic acid sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine.



38. The use of claim 37, wherein the nucleic acid sample in step (a) comprises mRNA corresponding to the transcript of DNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine, and wherein the determining of step (b) comprises:

- (i) contacting the mRNA with the oligonucleotide of claim 25 under conditions permitting binding of the mRNA to the oligonucleotide so as to form a complex;
- (ii) isolating the complex so formed; and
- (iii) identifying the mRNA in the isolated complex so as to thereby determine whether the mRNA is, or is derived from, a nucleic acid which encodes the polypeptide.

39. The use of claim 37, wherein the nucleic acid sample in step (a) comprises mRNA corresponding to the transcript of DNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine, and wherein the determining of step (b) comprises:

- (i) translating the mRNA under suitable conditions to obtain an amino acid sequence; and
- (ii) comparing the amino acid sequence of step (i) with the amino acid sequence of the isolated nucleic acid of claim 9 so as to thereby determine whether the nucleic acid sample is, or is derived from, a nucleic acid which encodes the polypeptide.



40. The use of claim 37, wherein the determining of step (b) comprises:
 - (i) amplifying the nucleic acid present in the sample of step (a); and
 - (ii) detecting the presence of polypeptide in the resulting amplified nucleic acid.
41. Use of an antibody that recognizes a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine for determining whether a subject is infected with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-133 Oon Strain (Methionine to Threonine), wherein such determination comprises:
 - (a) obtaining an appropriate sample from the subject; and
 - (b) determining whether the sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine by contacting the sample under appropriate conditions to bind to the antibodies of claim 35 or 36 so as to determine whether a subject is infected.
42. The use of claim 37, 38 or 41, wherein the isolated nucleic acid, oligonucleotide or antibody is labeled with a detectable marker.
43. The use the claim 42, wherein the detectable marker is a radioactive isotope, a fluorophor or an enzyme.



44. The use of claim 37, wherein the sample comprises blood, tissue or sera.
45. Use of a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine for determining whether a subject has a predisposition for hepatocellular carcinoma, which comprises:
 - (a) obtaining an appropriate nucleic acid sample from the subject; and
 - (b) determining whether the nucleic acid sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine.
46. The use of claim 45, wherein the nucleic acid sample in step (a) comprises mRNA corresponding to the transcript of DNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine, and wherein the determining of step (b), which comprises:
 - (i) contacting the mRNA with the oligonucleotide of claim 25 under conditions permitting binding of the mRNA to the oligonucleotide so as to form a complex;



- (ii) isolating the complex so formed; and
- (iii) identifying the mRNA in the isolated complex so as to thereby determine whether the mRNA is, or is derived from, a nucleic acid which encodes the polypeptide.

47. The use of claim 45, wherein the nucleic acid sample in step (a) comprises mRNA corresponding to the transcript of DNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine, and wherein the determining of step (b) comprises:

- (i) translating the mRNA under suitable conditions to obtain an amino acid sequence; and
- (ii) comparing the amino acid sequence of step (i) with the amino acid sequence of the isolated nucleic acid of claim 9 so as to thereby determine whether the nucleic acid sample is, or is derived from, a nucleic acid which encodes the polypeptide.

48. The use of claim 45, wherein the determining of step (b) comprises:

- (i) amplifying the nucleic acid present in the sample of step (a); and
- (ii) detecting the presence of polypeptide in the resulting amplified nucleic acid.

49. Use of an antibody that recognizes a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a



major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine for determining whether the subject has a predisposition for hepatocellular carcinoma, wherein such determination comprises:

- (a) obtaining an appropriate sample from the subject; and
- (b) determining whether the sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine by contacting the sample under appropriate conditions to bind to the antibodies of claim 35 or 36 so as to determine whether the subject subject has a predisposition for hepatocellular carcinoma.

- 50. The use of claim 46, 47 or 49, wherein the isolated nucleic acid, oligonucleotide or antibody is labeled with a detectable marker.
- 51. The use of claim 50, wherein the detectable marker is a radioactive isotope, a fluorophor or an enzyme.
- 52. The use of claim 45, wherein the sample comprises blood, tissue or sera.
- 53. A method for identifying a chemical compound for use in the manufacture of a medicament [which is] capable of treating infection by a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-133 Oon Strain (Methionine to Threonine) [which] wherein the method for identifying the chemical compound comprises:
 - (a) contacting a polypeptide which is a mutant major surface



antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine, with the chemical compound under conditions permitting binding between the polypeptide and the chemical compound;

- (b) detecting specific binding of the chemical compound to the polypeptide; and
- (c) determining whether the chemical compound inhibits the polypeptide so as to identify a chemical compound which is capable of treating infection by the viral strain.

54. A method for identifying a chemical compound for use in the manufacture of a medicament [which is] capable of preventing infection by a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-133 Oon Strain (Methionine to Threonine), [which] wherein the method for identifying the chemical compound comprises:

- (a) contacting a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine, with the chemical compound under conditions permitting binding between the polypeptide and the chemical compound;
- (b) detecting specific binding of the chemical compound to the polypeptide; and
- (c) determining whether the chemical compound inhibits the polypeptide so as to identify a chemical compound which is capable of preventing infection by the viral strain.

55. A method for identifying a chemical compound for use in the



manufacture of a medicament [which is] capable of treating hepatocellular carcinoma [which] wherein the method for identifying the chemical compound comprises:

- (a) contacting a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine, with the chemical compound under conditions permitting binding between the polypeptide and the chemical compound;
- (b) detecting specific binding of the chemical compound to the polypeptide; and
- (c) determining whether the chemical compound inhibits the polypeptide so as to identify a chemical compound which is capable of treating hepatocellular carcinoma.

56. A method for identifying a chemical compound for use in the manufacture of a medicament [which is] capable of preventing hepatocellular carcinoma, [which] wherein the method for identifying the chemical compound comprises:

- (a) contacting a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine, with the chemical compound under conditions permitting binding between the polypeptide and the chemical compound;
- (b) detecting specific binding of the chemical compound to the polypeptide; and
- (c) determining whether the chemical compound inhibits the polypeptide so as to identify a chemical compound which is capable of preventing infection by the viral strain.



57. A composition comprising a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine, [or derivative thereof,] the amounts of such polypeptide being effective to stimulate or enhance antibody production in a subject, and a pharmaceutically acceptable.
58. A composition comprising a peptide, wherein the peptide has an amino acid sequence comprising amino acid residues 298 through 320 of the amino acid sequence designated SEQ. I.D. No. 3. [or derivative thereof], the amounts of such peptide being effective to stimulate or enhance antibody production in a subject, and a pharmaceutically acceptable.
59. A composition comprising the chemical compound identified by the method of claim 55 in an amount effective to treat hepatocellular carcinoma and a pharmaceutically effective carrier.
60. A composition comprising the chemical compound identified by the method of claim 56 in an amount effective to prevent hepatocellular carcinoma and a pharmaceutically effective carrier.
61. A composition comprising the chemical compound identified by the method of claim 53 in an amount effective to treat a strain of hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-133 Oon Strain (Methionine to Threonine) and a pharmaceutically effective carrier.
62. A composition comprising the chemical compound identified by the method of claim 54 in an amount effective to prevent infection by a strain of hepatitis B virus designated Human



Hepatitis B Virus Surface Antigen-'S'-133 Oon Strain (Methionine to Threonine) and a pharmaceutically effective carrier.

63. Use of the composition of claim 57 or 58 for treating a subject infected with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-133 Oon Strain (Methionine to Threonine).
64. Use of the composition of claim 61 treating a subject infected with a strain of hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-133 Oon Strain (Methionine to Threonine).
65. Use of the composition of claim 57 or 58 for preventing infection by a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-133 Oon Strain (Methionine to Threonine) in a subject.
66. Use of the composition of claim 62 for preventing infection by a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-133 Oon Strain (Methionine to Threonine) in a subject.
67. Use of the composition of claim 57 or 58 for treating a subject with hepatocellular carcinoma.
68. Use of the composition of claim 59 for treating a subject with hepatocellular carcinoma.
69. Use of the composition of claim 57 or 59 for preventing hepatocellular carcinoma in a subject.
70. Use of the composition of claim 60 for preventing hepatocellular carcinoma in a subject.



71. A method of screening bodily fluids from a subject for a strain of hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-133 Oon Strain (Methionine to Threonine) which comprises:
 - (a) obtaining an appropriate sample of bodily fluid from the subject;
 - (b) determining the presence of a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 133 of such polypeptide is a threonine, rather than a methionine in the sample of step (a) so as to screen the sample for the strain.
72. The method of claim 71, wherein the bodily fluid comprises blood, sera, or a nucleic acid sample of blood or sera.
73. A hepatitis vaccine, comprising a mutant form of the surface antigen of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the wildtype amino acid sequence of the major surface antigen of hepatitis B in that the amino acid at position number 133 of such polypeptide is a threonine rather than a methionine.
74. The vaccine of claim 73, further comprising an adjuvant.



PATENT COOPERATION TREATY

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

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| International application No. PCT/SG98/00045 | Applicant's or agent's file reference PCT/8013374 |
| International filing date (day/month/year) 19 June 1998 (19.06.98) | Priority date (day/month/year) |
| Applicant OON, Chong, Jin et al | |

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:

18 January 2000 (18.01.00)

☐ in a notice effecting later election filed with the International Bureau on:2. The election ☒ was☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

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| <p>The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland</p> <p>Facsimile No.: (41-22) 740.14.35</p> | <p>Authorized officer F. Baechler</p> <p>Telephone No.: (41-22) 338.83.38</p> |
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What is claimed is:

1. An isolated strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) which constituent viral genome is deposited under Accession Nos. P97121504, P97121505 and P97121506 with the European Collection of Cell Culture on 15th December 1997.
2. An isolated nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine.
3. The isolated nucleic acid of claim 2, wherein the polypeptide is being encoded by nucleotides 155 through 835 of the nucleic acid sequence designated SEQ. I.D. No. 1.
4. The isolated nucleic acid of claim 3, comprising nucleotides "AGA" in positions 587-589.
5. The isolated nucleic acid of claim 2, wherein the nucleic acid is DNA.
6. The isolated nucleic acid of claim 2, wherein the nucleic acid is RNA.
7. The isolated nucleic acid of claim 5, wherein the nucleic acid is cDNA.
8. The isolated nucleic acid of claim 5, wherein the nucleic acid is genomic DNA.



9. The isolated nucleic acid of claim 2, wherein the polypeptide has an amino acid sequence substantially the same as amino acid residues 174 through 400 of the amino acid sequence designated SEQ. I.D. No. 3.
- 5
10. An isolated nucleic acid which encodes a peptide, wherein the peptide is encoded by a nucleic acid molecule comprising nucleotides 527 through 595 of SEQ. I.D. No. 1.
- 10
11. An isolated nucleic acid which encodes a peptide, wherein the peptide has an amino acid sequence comprising amino acid residues 298 through 320 of the amino acid sequence designated SEQ. I.D. No. 3.
- 15
12. A vector comprising an isolated nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine and operatively linked to a promoter of RNA transcription.
- 20
13. A vector comprising an isolated nucleic acid encoding a peptide, wherein the peptide is encoded by a nucleic acid molecule comprising nucleotides 527 through 595 of SEQ. I.D. No. 1.
- 25
14. The vector of claim 12 or 13, wherein the vector comprises viral DNA.
- 30
15. A host vector system for the production of a polypeptide which comprises the vector of claim 12 in a suitable host.
- 35
16. A host vector system for the production of a peptide



which comprises the vector of claim 13 in a suitable host.

17. A method of producing a polypeptide which comprises
5 growing the host vector system of claim 15 under suitable conditions permitting production of the polypeptide and recovering the polypeptide so produced.
18. A method of producing a peptide which comprises growing
10 the host vector system of claim 16 under suitable conditions permitting production of the polypeptide and recovering the polypeptide so produced.
19. A method of obtaining a polypeptide in purified form
15 which comprises:
 (a) introducing the vector of claim 12 into a suitable host cell;
 (b) culturing the resulting host cell so as to produce the polypeptide;
20 (c) recovering the polypeptide produced in step (b);
 and
 (d) purifying the polypeptide so recovered.
20. A method of obtaining a polypeptide in purified form
25 which comprises:
 (a) introducing the vector of claim 13 into a suitable host cell;
 (b) culturing the resulting host cell so as to produce the polypeptide;
30 (c) recovering the polypeptide produced in step (b);
 and
 (d) purifying the polypeptide so recovered.
21. A purified polypeptide which is a mutant major surface
35 antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid



at position number 145 of such polypeptide is an arginine rather than a glycine.

22. A purified polypeptide obtained from the method of claim 19.
23. A purified peptide, wherein the peptide has an amino acid sequence comprising amino acid residues 298 through 320 of the amino acid sequence designated SEQ. I.D. No. 3.
24. A purified peptide obtained from the method of claim 20.
25. An oligonucleotide of at least 15 nucleotides capable of specifically hybridizing with a unique sequence of nucleotides within a nucleic acid which encodes a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine, without hybridizing to any sequence of nucleotides within a nucleic acid which encodes the major surface antigen of a wild type hepatitis B virus.
26. The oligonucleotide of claim 25 comprising nucleotides 527 through 595 of SEQ. I.D. No. 1.
27. A method of obtaining antibodies to a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and not to the major surface antigen of a wild type hepatitis B virus, comprising:



- (a) obtaining the polypeptide in a purified form;
 - (b) immunizing an organism capable of producing antibodies against the purified polypeptide;
 - (c) collecting the produced antibodies;
 - 5 (d) combining the produced antibodies and the purified polypeptide under conditions to form a complex; and
 - (e) determining which produced antibodies form a complex with the purified polypeptide so as to obtain antibodies to the polypeptide.
- 10 28. The method of claim 27, wherein the polypeptide is being encoded by nucleotides 155 through 835 of the nucleic acid sequence designated SEQ. I.D. No. 1.
- 15 29. The method of claim 27, wherein the polypeptide has an amino acid sequence substantially the same as amino acid residues 174 through 400 of the amino acid sequence designated SEQ. I.D. No. 3.
- 20 30. The method of claim 27, wherein the organism comprises a rabbit or a mouse.
31. A method of obtaining antibodies to a peptide, wherein the peptide has an amino acid sequence comprising amino acid residues 298 through 320 of the amino acid sequence designated SEQ. I.D. No. 3, comprising:
- 25 (a) obtaining the peptide in a purified form;
- (b) immunizing an organism capable of producing antibodies against the purified peptide;
- 30 (c) collecting the produced antibodies;
- (d) combining the produced antibodies and the purified peptide under conditions to form a complex; and
- (e) determining which produced antibodies form a complex with the purified peptide so as to obtain antibodies to the peptide.
- 35 32. The method of claim 31, wherein the organism comprises a rabbit or a mouse.



33. The antibodies obtained in claim 27 or 31.
34. Monoclonal antibodies of the antibodies of claim 33.
- 5 35. Antibodies capable of detecting a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that
10 the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and incapable of detecting the major surface antigen of a wild type hepatitis B virus.
- 15 36. Antibodies capable of detecting a peptide, wherein the peptide has an amino acid sequence comprising amino acid residues 298 through 320 of the amino acid sequence designated SEQ. I.D. No. 3.
- 20 37. Use of a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that
25 the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine for determining whether a subject is infected with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine),
30 wherein such determination comprises
(a) obtaining an appropriate nucleic acid sample from the subject; and
(b) determining whether the nucleic acid sample from
35 step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a



major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine.

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38. The use of claim 37, wherein the nucleic acid sample in step (a) comprises mRNA corresponding to the transcript of DNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and wherein the determining of step (b) comprises:

15

- (i) contacting the mRNA with the oligonucleotide of claim 25 under conditions permitting binding of the mRNA to the oligonucleotide so as to form a complex;
- (ii) isolating the complex so formed; and
- (iii) identifying the mRNA in the isolated complex so as to thereby determine whether the mRNA is, or is derived from, a nucleic acid which encodes the polypeptide.

25

39. The use of claim 37, wherein the nucleic acid sample in step (a) comprises mRNA corresponding to the transcript of DNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and wherein the determining of step (b) comprises:

35

- (i) translating the mRNA under suitable conditions to obtain an amino acid sequence; and
- (ii) comparing the amino acid sequence of step (i)



with the amino acid sequence encoded by the isolated nucleic acid of claim 9 so as to determine whether the nucleic acid sample is, or is derived from, a nucleic acid which encodes the polypeptide.

40. The use of claim 37, wherein the determining of step (b) comprises:

- (i) amplifying the nucleic acid present in the sample of step (a); and
- (ii) detecting the presence of polypeptide in the resulting amplified nucleic acid.

41. Use of antibodies capable of detecting a polypeptide which is a mutant major surface antigen of a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) for determining whether a subject is infected with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine), wherein such determination comprises:

- (a) obtaining an appropriate sample from the subject; and

- (b) determining whether the sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, by contacting the sample under appropriate conditions to bind to the antibodies of claim 35 or 36 so as to determine whether a subject is infected.

42. The use of claim 37, 38 or 41, wherein the isolated



nucleic acid, oligonucleotide, or antibody is labeled with a detectable marker.

43. The use the claim 42, wherein the detectable marker is
5 a radioactive isotope, a fluorophor, or an enzyme.
44. The use of claim 37, wherein the sample comprises blood,
tissue, or sera.
- 10 45. A method for identifying a chemical compound for the
manufacture of a medicament which is capable of treating
infection by a strain of Hepatitis B virus designated
Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore
Strain (Glycine to Arginine), which comprises:
- 15 (a) contacting a polypeptide which is a mutant major
surface antigen of a strain of hepatitis B virus,
such polypeptide having an amino acid sequence
which differs from the amino acid sequence of a
major surface antigen of a wild type hepatitis B
20 virus in that the amino acid at position number 145
of such polypeptide is an arginine, rather than a
glycine, with the chemical compound under
conditions permitting binding between the
polypeptide and the chemical compound;
- 25 (b) detecting specific binding of the chemical compound
to the polypeptide; and
- (c) determining whether the chemical compound binds to
the polypeptide so as to identify a chemical
compound which is capable of treating infection by
30 the viral strain.
46. A method for identifying a chemical compound for the
manufacture of a medicament which is capable of
preventing infection by a strain of Hepatitis B virus
35 designated Human Hepatitis B Virus Surface Antigen-'S'-
145 Singapore Strain (Glycine to Arginine), which
comprises:
- (a) contacting a polypeptide which is a mutant major



5 surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, with the chemical compound under conditions permitting binding between the polypeptide and the chemical compound;

- 10 (b) detecting specific binding of the chemical compound to the polypeptide; and
- (c) determining whether the chemical compound binds to the polypeptide so as to identify a chemical compound which is capable of preventing infection
- 15 by the viral strain.

47. A composition comprising a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which

20 differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, or derivative thereof, the amounts of such polypeptide being effective

25 to stimulate or enhance antibody production in a subject, and a pharmaceutically acceptable carrier.

48. A composition comprising a peptide, wherein the peptide has an amino acid sequence comprising amino acid residues

30 298 through 320 of the amino acid sequence designated SEQ. I.D. No. 3. or derivative thereof, the amounts of such peptide being effective to stimulate or enhance antibody production in a subject, and a pharmaceutically acceptable carrier.

35 49. A composition comprising the chemical compound identified by the method of claim 45 in an amount effective to treat infection by a strain of Hepatitis B virus designated



Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) and a pharmaceutically effective carrier.

50. A composition comprising the chemical compound identified by the method of claim 46 in an amount effective to prevent infection by a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) and a pharmaceutically effective carrier.
1. Use of the composition of claim 47 or 48 for treating a subject infected with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine).
52. Use of the composition of claim 45 for treating a subject infected with a strain of hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine).
- 20
53. Use of the composition of claim 47 or 48 for preventing infection by a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) in a subject.
- 25
54. Use of the composition of claim 46 for preventing infection with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) in a subject.
- 30
55. A method of screening bodily fluids from a subject for a strain of hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) which comprises:
- 35
- (a) obtaining an appropriate sample of bodily fluid from the subject;
 - (b) determining the presence of a polypeptide which is



a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, in the sample of step (a) so as to screen the sample for the strain.

56. The method of claim 55, wherein the bodily fluid comprises blood, sera, or a nucleic acid sample of blood or sera.
57. Use of an antibody that recognizes a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus for determining whether the subject has a predisposition for hepatocellular carcinoma, wherein such determination comprises:
- (a) obtaining an appropriate nucleic acid sample from the subject; and
 - (b) determining whether the nucleic acid sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, by contacting the sample under appropriate conditions to bind to the antibodies of claim 35 so as to determine whether the subject has a predisposition for hepatocellular carcinoma.
58. The method of claim 57, wherein the nucleic acid sample in step (a) comprises mRNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid



sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and wherein the determining of step (b) comprises:

- (i) contacting the mRNA with the oligonucleotide of claim 25 under conditions permitting binding of the mRNA to the oligonucleotide so as to form a complex;
- (ii) isolating the complex so formed; and
- (iii) identifying the mRNA in the isolated complex so as to thereby determine whether the mRNA is, or is derived from, a nucleic acid which encodes the polypeptide.

59. The method of claim 57, wherein the nucleic acid sample in step (a) comprises mRNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and wherein the determining of step (b) comprises:

- (i) translating the mRNA under suitable conditions to obtain an amino acid sequence; and
- (ii) comparing the amino acid sequence of step (i) with the amino acid sequence encoded by the isolated nucleic acid of claim 9 so as to determine whether the nucleic acid sample is, or is derived from, a nucleic acid which encodes the polypeptide.

60. The method of claim 57, wherein the determining of step (b) comprises:

- (i) amplifying the nucleic acid present in the sample of step (a); and
- (ii) detecting the presence of polypeptide in the



resulting amplified nucleic acid.

61. Use of an antibody that recognizes a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus for determining whether the subject has a predisposition for hepatocellular carcinoma, wherein such determination comprises:
- (a) obtaining an appropriate sample from the subject; and
 - (b) determining whether the sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, by contacting the sample under appropriate conditions to bind to the antibodies of claim 36 so as to determine whether the subject has a predisposition for hepatocellular carcinoma.
62. The method of claim 58, 59 or 61, wherein the oligonucleotide or antibody is labeled with a detectable marker.
63. The method of claim 62, wherein the detectable marker is a radioactive isotope, a fluorophor or an enzyme.
64. The method of claim 57, wherein the sample comprises blood, tissue or sera.
65. A method for identifying a chemical compound for the manufacture of a medicament which is capable of treating hepatocellular carcinoma which comprises:
- (a) contacting a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus,



such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, with the chemical compound under conditions permitting binding between the polypeptide and the chemical compound;

- (b) detecting specific binding of the chemical compound to the polypeptide; and
- (c) determining whether the chemical compound binds to the polypeptide so as to identify a chemical compound which is capable of treating hepatocellular carcinoma.

66. A method for identifying a chemical compound for the manufacture of a medicament which is capable of preventing hepatocellular carcinoma, which comprises:

- (a) contacting a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, with the chemical compound under conditions permitting binding between the polypeptide and the chemical compound;
- (b) detecting specific binding of the chemical compound to the polypeptide; and
- (c) determining whether the chemical compound binds to the polypeptide so as to identify a chemical compound which is capable of preventing hepatocellular carcinoma.

67. A composition comprising the chemical compound identified by the method of claim 65 in an amount effective to treat hepatocellular carcinoma and a pharmaceutically effective





carrier.

68. A composition comprising the chemical compound identified
by the method of claim 66 in an amount effective to
5 prevent hepatocellular carcinoma and a pharmaceutically
effective carrier.
69. Use of the composition of claim 47 or 48 as a medicament
for treating hepatocellular carcinoma.
- 10 70. Use of the composition of claim 67 as a medicament for
treating hepatocellular carcinoma.
71. Use of the composition of claim 47 or 48 as a medicament
15 for preventing hepatocellular carcinoma.
72. Use of the composition of claim 67 as a medicament for
preventing hepatocellular carcinoma.
- 20 73. A hepatitis vaccine, comprising a mutant form of the
surface antigen of hepatitis B virus, such polypeptide
having an amino acid sequence which differs from the
amino acid sequence of the major surface antigen of
hepatitis B in that the amino acid at position number 145
25 of such polypeptide is an arginine rather than a glycine.
74. The vaccine of claim 73, further comprising an adjuvant.



INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

| | | | |
|--|--|---|--|
| Applicant's or agent's file reference CG/PHM/PAT/8013374 | | See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) FOR FURTHER ACTION | |
| International application No PCT/SG98/00045 | International filing date (day/month/year) 19/06/1998 | Priority date (day/month/year) 19/06/1998 | |
| International Patent Classification (IPC) or national classification and IPC C12N15/51 | | | |
| Applicant GOVERNMENT OF REPUBLIC OF SINGAPORE ... et al. | | | |
| <p>1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 6 sheets, including this cover sheet.</p> <p><input checked="" type="checkbox"/> This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).</p> <p>These annexes consist of a total of 17 sheets.</p> | | | |
| <p>3. This report contains indications relating to the following items:</p> <ul style="list-style-type: none"> I <input checked="" type="checkbox"/> Basis of the report II <input type="checkbox"/> Priority III <input type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability IV <input type="checkbox"/> Lack of unity of invention V <input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement VI <input type="checkbox"/> Certain documents cited VII <input checked="" type="checkbox"/> Certain defects in the international application VIII <input checked="" type="checkbox"/> Certain observations on the international application | | | |
| Date of submission of the demand 18/01/2000 | | Date of completion of this report 15.01.00 | |
| Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465 | | Authorized officer SCHEFFZYK, I Telephone No. +49 89 2399 8602  | |



**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/SG98/00045

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.)*:

Description, pages:

1-35 as originally filed

Claims, No.:

1-74 as received on 13/06/2000 with letter of 09/06/2000

Drawings, sheets:

1/2.2/2 as originally filed

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:



**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/SG98/00045

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

| | | | |
|-------------------------------|------|--------|-----------------------------|
| Novelty (N) | Yes: | Claims | 1-26,35-74 |
| | No: | Claims | 27-34 |
| Inventive step (IS) | Yes: | Claims | 1-26,35-74 |
| | No: | Claims | 27-34 |
| Industrial applicability (IA) | Yes: | Claims | 1-50,55-74 |
| | No: | Claims | 51-54: see section VIII/8). |

2. Citations and explanations

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet



**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/SG98/00045

SECTION V-----

The subject-matter of claims 1-26, 35-74 is deemed novel and inventive since the existence of the Hepatitis B virus strain according to claim 1 which is characterized in that the surface major antigen contains at amino acid position 145 arginine in place of glycine was neither described nor derivable from the available prior art.

Thus, these claims meet the requirements of Art. 33(2)(3) PCT.

However, for the reasons set out below in section VIII(2), claims 27-34 cannot be considered to be novel over readily available antibodies directed against Hepatitis B viruses and methods of producing them.

Correspondingly, these claims do not comply with the requirements of Art. 33(2)(3) PCT.

SECTION VII-----

- 1). With respect to the expression "incorporated by reference" written in the specification Applicant's attention is drawn to Guidelines C-II 4.3 and C-II 4.18 PCT.

SECTION VIII-----

- 1). For the sake of clarity claim 1 should contain a reference to a SEQ.ID.NO.
- 2). With respect to claims 27-32 it is noted that it is questionable whether the methods defined in these claims actually contain all features essential to obtain antibodies exclusively binding to the mutated major surface antigen of hepatitis B virus which only differs from the corresponding wild-type protein in that it has arginine at position 145 instead of glycine. Relating to this it is noted that step e) defined in claims 27 and 31 also covers the detection and correspondingly also the provision of antibodies binding to regions of the wild-type major surface antigen! Moreover, the residues defined in claims 29 and 31 correspond to those of the wild-type major surface antigen.



**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/SG98/00045

- 3). The scope of claims 45, 46, 65, 66 is unclear since it is not known whether said claims are directed to a method of identifying a chemical compound or whether they are directed to the use of chemical compounds in a method for the manufacture of a medicament.
- 4). The term "derived from" used in claim 57 renders the scope of said claim unclear (Art. 6 PCT).
- 5). Claims 49, 50, 67 and 68 are not supported by the specification since the specification fails to mention any chemical compound identified by the method according to claims 45, 46, 65 and 66 (Art. 6 PCT in combination with Guidelines C-III 6.3 PCT). Moreover, with respect to these claims an objection under Art. 5 PCT also arises since in the absence of evidence showing that chemical compounds suitable to exclusively bind to the mutant major surface antigen having at position 145 arginine in place of glycine actually exist the existence of such compounds is merely speculative and thus it is questionable whether a person skilled in the art actually is able to prepare the claimed compositions.
- 6). The same objections apply equally to claims 52 and 46 relating to the use of the compositions according to claims 49, 50, 67 and 68.
- 7). The reference in claims 52, 54, 70 and 72 should be checked (claims 45, 46 and 67 are not directed to compositions but to methods).
- 8). Claims 51-54, 57-64, 69-72 are not supported by the specification since the application as originally filed fails to provide any facts and data demonstrating that the mutant form (Arg instead of Gly at position 145) of the major surface antigen is actually suitable for the medical applications defined in these claims. Relating to this it is also pointed out that there is no evidence in the application showing a relationship of the occurrence of said mutant form with the development of HCC (hepatocellular carcinoma).
- 9). Claims 51-54 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these



**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/SG98/00045

claims (Article 34(4)(a)(i) PCT).



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29 February 2000 (29.02.00)Applicant's or agent's file reference
PCT/8013374

IMPORTANT INFORMATION

International application No.
PCT/SG98/00045International filing date (day/month/year)
19 June 1998 (19.06.98)

Priority date (day/month/year)

Applicant

GOVERNMENT OF THE REPUBLIC OF SINGAPORE et al

1. The applicant is hereby informed that the International Bureau has, according to Article 31(7), notified each of the following Offices of its election:

EP : AT, BE, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, NL, PT, SE

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2. The following Offices have waived the requirement for the notification of their election; the notification will be sent to them by the International Bureau only upon their request:

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3. The applicant is reminded that he must enter the "national phase" **before the expiration of 30 months from the priority date** before each of the Offices listed above. This must be done by paying the national fee(s) and furnishing, if prescribed, a translation of the international application (Article 39(1)(a)), as well as, where applicable, by furnishing a translation of any annexes of the international preliminary examination report (Article 36(3)(b) and Rule 74.1).

Some offices have fixed time limits expiring later than the above-mentioned time limit. For detailed information about the applicable time limits and the acts to be performed upon entry into the national phase before a particular Office, see Volume II of the PCT Applicant's Guide.

The entry into the European regional phase is postponed **until 31 months from the priority date** for all States designated for the purposes of obtaining a European patent.

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| (21) International Application Number: PCT/SG98/00045 (22) International Filing Date: 19 June 1998 (19.06.98) (71) Applicant (for all designated States except US): GOVERNMENT OF THE REPUBLIC OF SINGAPORE [SG/SG]; Ministry of Health, College of Medicine Building, 18 College Road, Singapore 169854 (SG). (72) Inventors; and (75) Inventors/Applicants (for US only): OON, Chong, Jin [SG/SG]; 14A Princess of Wales Road, Singapore 266 914 (SG). LIM, Gek, Keow [SG/SG]; 16 Telok Kurau, Lorong G, Singapore 426180 (SG). LEONG, Ai, Lin [SG/SG]; Block 263, Yishun Street 22 #12-161, Singapore 760263 (SG). ZHAO, Yi [SG/SG]; Block 345, Bukit Batok Street 34 #12-270, Singapore 650345 (SG). CHEN, Wei, Ning [SG/SG]; Block 104, Spottiswoode Park Road #22-114, Singapore 080104 (SG). (74) Agent: DREW & NAPIER; 20 Raffles Place, #17-00 Ocean Towers, Singapore 048620 (SG). | | (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> |
| (54) Title: A VACCINE-INDUCED HEPATITIS B VIRAL STRAIN AND USES THEREOF (57) Abstract This invention provides an isolated strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen- \dot{S} -145 Singapore Strain (Glycine to Arginine) which constituent viral genome is deposited under Accession Nos. P97121504, P97121505 and P97121506 with the European Collection of Cell Culture on 15th December 1997. This invention also provides an isolated nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine, and the purified peptide. This invention further provides an isolated nucleic acid which encodes a peptide, wherein the peptide is encoded by a nucleic acid molecule comprising nucleotides 527 through 595 of SEQ. ID. No. 1, and the purified peptide. This invention also provides various methods using the disclosed isolated nucleic acids and polypeptides. This invention also provides various uses of the viral strain and its proteins. | | |



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528 B-14-10-10-10-10-10
A VACCINE-INDUCED HEPATITIS B
VIRAL STRAIN AND USES THEREOF

Throughout this application, various references are referred
5 to within parentheses. Disclosures of these publications in
their entireties are hereby incorporated by reference into
this application to more fully describe the state of the art
to which this invention pertains.

10 BACKGROUND OF THE INVENTION

The present invention concerns the hepatitis B virus genome
with a vaccine-induced mutation at amino acid residue 145
(Glycine to Arginine) within the major surface antigen, its
15 nucleotide sequence, the deduced four major protein
sequences, antigen, antibody, detection systems, development
of effective vaccines, and antiviral agents.

The hepatitis B virus was first discovered in 1963 as a human
20 virus that is transmitted parenterally. Although these
viruses are not particularly cytotoxic and do not lead to
massive cell death, they have been the cause of a major
infectious disease affecting both adults and young children
worldwide. The presence of hepatitis B surface antigen has
25 served as the main detection marker for carriers of hepatitis
B virus, and thus, possibly those at risk of transmitting the
virus. Conversely, the occurrence of an anti-surface antigen
antibody indicates an immune response which would lead to
eventual recovery. Stimulation of such immune response has
30 been greatly helped by the currently licensed hepatitis B
vaccines developed by Merck Sharpe & Dohme. These vaccines
contain the major surface antigen in either the natural
(plasma-derived) or the recombinant (purified from yeast
cells) form, and have proven safe and effective in
35 neutralizing the hepatitis B virus. In Singapore, the active
vaccination program at a national scale has resulted in a
significant decrease of acute hepatitis B infection and the
incidence of primary hepatocellular carcinoma. This decrease
has in turn been associated with an increased immunity in the
40 population.

The major antigenic epitope of hepatitis B virus is a highly conserved region spanning 23 amino acid residues and located from amino acid position 124 to 147 of the major surface antigen. This small region designated as the group specific
5 determinant "a" is found in all subtypes and isolates of hepatitis B viral genomes. Its antigenic properties seem due to its proposed double loop structure, to which the vaccine-induced neutralizing antibody binds.

10 In contrast to random mutations introduced into hepatitis B viral genomes during viral replication by the proof-reading defective reverse transcriptase, mutations induced following vaccination occur mainly in the "a" epitope of the major surface antigen. These mutant viruses are of particular
15 interest since they show reduced affinity to the neutralizing antibody and therefore are able to replicate independently. Among these vaccine-escape mutants, the mutation at amino acid residue 145 (from Glycine to Arginine) in the second loop of the major surface antigen is the most significant
20 because it is stable, results in conformational changes of the "a" epitope and has been reported worldwide in North America, Europe, Japan and Southeast Asia. In Singapore, for example, such mutants are the most frequent variant following vaccination. Twelve infectious variants among 41
25 breakthroughs have been identified as having an arginine mutation at amino acid residue 145 of the major surface antigen. There is evidence of vertical transmission from one of the 12 variants and this variant has also been associated with active liver disease. Significantly, some of these
30 variants are now found in random asymptomatic adult population.

The occurrence of this replicative vaccine-induced mutant and its ability to escape detection using standard reagents is of
35 grave concern because it has resulted in the development of acute hepatitis B in Italy and Singapore. This situation therefore requires the urgent development of specific detection systems, as well as, effective prophylactic

vaccines and antiviral agents. Determination of the nucleotide sequence of this vaccine-induced mutant virus constitutes the first step towards these aims and will certainly be helpful for the various above-mentioned
s developments.

SUMMARY OF THE INVENTION

This invention provides an isolated strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-
5 145 Singapore Strain (Glycine to Arginine) which constituent viral genome is deposited under Accession Nos. P97121504, P97121505 and P97121506 with the European Collection of Cell Culture on 15th December 1997.

10 This invention also provides an isolated nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type
15 hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine.

This invention provides a method of producing the polypeptide in purified form and the resulting purified
20 polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such
25 polypeptide is an arginine rather than a glycine.

This invention provides an oligonucleotide of at least 15 nucleotides capable of specifically hybridizing with sequences of only the mutant viral strain of hepatitis B
30 virus.

This invention provides a method of obtaining antibodies to a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus and the antibodies produced.

35 This invention provides uses of the above-described polypeptide, antibodies or nucleic acid for determining whether a subject is infected with the above-described viral

strain.

This invention also provides a composition capable of stimulating or enhancing antibody production for the
5 polypeptide.

This invention further provides a method for identifying a chemical compound which is capable of treating and/or preventing an infection by the above-described mutant viral
10 strain and compositions containing such compounds.

This invention also provides a composition comprising the chemical compound identified by the above-described methods in an amount effective to treat or prevent infection by the
15 strain and a pharmaceutically effective carrier.

This invention further provides use of compositions for treating a subject infected with this viral strain.

20 This invention also provides use of compositions for preventing infection of a subject by this viral strain.

And lastly, this invention provides a method of screening bodily fluids for this viral strain.

BRIEF DESCRIPTION OF THE FIGURES

- Figure 1. Structure of the four open reading frames of human hepatitis B viral genome isolated from a male, eleven year old Singaporean child with a glycine to arginine mutation at amino acid residue 145 of the major surface antigen, as labeled by an asterisk. The major viral proteins: DNA polymerase, large/middle/major surface antigen, precore, core and transactivating X are denoted as P, PreS 1/PreS2/S, PreC, C and X respectively.
- Figure 2. Strategy of cloning and sequence determination of the same hepatitis B viral genome.
- Figure 3. Whole nucleotide sequence of human hepatitis B virus, isolated from an eleven year old child born to a mother in Singapore with the wild type virus. The child had received standard Hepatitis B immunoglobulin (HBIG) and HB vaccine and was infected with the mutated strain one year later. This strain carries a mutation at amino acid residue 145 (glycine to arginine) of the major surface antigen (SEQ. I.D. No. 1). The mutation is shown at nucleic acids numbered 587-589.
- Figure 4. Deduced amino acid sequence of the DNA polymerase from the nucleotide sequence of Figure 3 (SEQ. I.D. No. 2).
- Figure 5. Deduced amino acid sequence of the large surface antigen from the nucleotide sequence of Figure 3. The mutated amino acid residue (G to R) is numbered 319 (SEQ. I.D. No. 3).

- Figure 6. Deduced amino acid sequence of the core protein from the nucleotide sequence of Figure 3 (SEQ. I.D. No. 4).
- 5 Figure 7. Deduced amino acid sequence of the trans-activating X protein from the nucleotide sequence of Figure 3 (SEQ. I.D. No. 5).
- 10 Figure 8. Oligonucleotide sequence corresponding to the initiation site of the coding region of DNA polymerase, at position 2307 of the viral genome and matches the coding strand (sense oligonucleotide) (SEQ.I.D.No.6).
- 15 Figure 9. Oligonucleotide sequence corresponding to position 250 of the viral nucleotide sequence and matches the complementary strand (anti-sense oligonucleotide) (SEQ.I.D.No.7).
- 20 Figure 10. Oligonucleotide sequence corresponding to position 250 of the viral nucleotide sequence and matches the coding strand (sense oligonucleotide) (SEQ.I.D.No.8).
- 25 Figure 11. Oligonucleotide sequence corresponding to the stop codon of the coding region of DNA polymerase, at position 1623 of the viral genome and matches the complementary strand (anti-sense oligonucleotide) (SEQ.I.D.No.9).
- 30 Figure 12. Oligonucleotide sequence corresponding to position 1420 of the viral genome and matches the coding strand (sense oligonucleotide) (SEQ.I.D.No.10).
- 35 Figure 13. Oligonucleotide sequence corresponding to position 2340 of the viral genome and matches

the complementary strand (anti-sense
oligonucleotide) (SEQ.I.D.No.11).

DETAILED DESCRIPTION OF THE INVENTION

Throughout this application, references to specific nucleotides are to nucleotides present on the coding strand
5 of the nucleic acid. The following standard abbreviations are used throughout the specification to indicate specific nucleotides:

| | | |
|----|-------------|-------------|
| | C=cytosine | A=adenosine |
| 10 | T=thymidine | G=guanosine |

The present invention provides the nucleotide sequence of a hepatitis B virus genome, which carries a vaccine-induced mutation at amino acid residue 145 (Glycine to Arginine) of
15 the major surface antigen, consisting of 3215 nucleotides (Figure 3) coding for 4 overlapping viral proteins shown in Figures 4-7.

The invention provides amino acid sequences of the four major
20 viral proteins, these include the DNA polymerase, large/middle/major surface antigen, core and trans-activating X. These proteins can be produced using recombinant technology, and used in developing polyclonal or monoclonal antibodies.

25 The present invention also provides a hepatitis B virus diagnostic system, specific for the vaccine-induced mutation at amino acid residue 145 (Glycine to Arginine) of the major surface antigen, using nucleotide or protein sequences or
30 antibodies described herein.

The present invention provides an isolated strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) which
35 constituent viral genome is deposited under Accession Nos. P97121504, P97121505 and P97121506.

The invention also provides an isolated nucleic acid encoding

a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of the major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine. In a specific embodiment, the polypeptide is being encoded by nucleotides 155 through 835 of the nucleic acid sequence designated SEQ. I.D. No. 1, specifically, comprising nucleotides "AGA" in position 587-589, instead of "GGA." This nucleic acid can be DNA or RNA, specifically, cDNA or genomic DNA.

In another embodiment of the invention, the polypeptide has an amino acid sequence substantially the same as amino acid residues 174 through 400 of the amino acid sequence designated SEQ. I.D. No. 3.

This invention further provides an isolated nucleic acid which encodes a peptide, wherein the peptide is encoded by a nucleic acid molecule comprising nucleotides 527 through 595 of SEQ. I.D. No. 1.

This invention also provides an isolated nucleic acid which encodes a peptide, wherein the peptide has an amino acid sequence comprising amino acid residues 298 through 320 of the amino acid sequence designated SEQ. I.D. No. 3.

This invention also provides a vector comprising an isolated nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine and operatively linked to a promoter of RNA transcription.

Further, this invention provides a vector comprising an isolated nucleic acid encoding a peptide, wherein the peptide is encoded by a nucleic acid molecule comprising nucleotides 527 through 595 of SEQ. I.D. No. 1.

5

In both of the above-identified vectors, the vector may comprise viral DNA.

10 This invention also provides a host vector system for the production of a polypeptide which comprises the above-described vectors in a suitable host.

15 This invention also provides a method of producing a polypeptide or a peptide which comprises growing the host vector systems described above, under suitable conditions permitting production of the polypeptide and recovering the polypeptide so produced.

20 This invention further provides a method of obtaining a polypeptide or a peptide in purified form which comprises: (a) introducing the above-described vectors into a suitable host cell; (b) culturing the resulting host cell so as to produce the polypeptide; (c) recovering the polypeptide produced in step (b); and (d) purifying the polypeptide so
25 recovered.

30 This invention further provides a purified polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine. One means of obtaining the polypeptide is by the above-described method.

35

This invention also provides a purified peptide, wherein the peptide has an amino acid sequence comprising amino acid residues 298 through 320 of the amino acid sequence

designated Seq. I.D. No. 3. One means of obtaining this peptide is by the above-described method.

This invention also provides an oligonucleotide of at least
5 15 nucleotides capable of specifically hybridizing with a
unique sequence of nucleotides within a nucleic acid which
encodes a polypeptide which is a mutant major surface antigen
of a strain of hepatitis B virus, such polypeptide having an
amino acid sequence which differs from the amino acid
10 sequence of a major surface antigen of a wild type hepatitis
B virus in that the amino acid at position number 145 of such
polypeptide is an arginine rather than a glycine, without
hybridizing to any sequence of nucleotides within a nucleic
acid which encodes the major surface antigen of a wild type
15 hepatitis B virus. Specifically the oligonucleotide comprises
nucleotides 527 through 595 of SEQ. I.D. No. 1.

This invention also provides a method of obtaining antibodies
to a polypeptide which is a mutant major surface antigen of
20 a strain of hepatitis B virus, such polypeptide having an
amino acid sequence which differs from the amino acid
sequence of a major surface antigen of a wild type hepatitis
B virus in that the amino acid at position number 145 of such
polypeptide is an arginine, rather than a glycine, and not to
25 the major surface antigen of a wild type hepatitis B virus,
comprising: (a) obtaining the polypeptide in a purified form;
(b) immunizing an organism capable of producing antibodies
against the purified polypeptide; (c) collecting the produced
antibodies; (d) combining the produced antibodies and the
30 purified polypeptide under conditions to form a complex; and
(e) determining which produced antibodies form a complex with
the purified polypeptide so as to obtain antibodies to the
polypeptide. Specifically, the polypeptide is being encoded
by nucleotides 155 through 835 of the nucleic acid sequence
35 designated SEQ. I.D. No. 1. In another embodiment, the
polypeptide has an amino acid sequence substantially
identical to amino acid residues 174 through 400 of the amino
acid sequence designated SEQ. I.D. No. 3.

One could perform the above-described well-known method in rabbits or mice.

This invention also provides a method of obtaining antibodies to a peptide, wherein the peptide has an amino acid sequence comprising amino acid residues 298 through 320 of the amino acid sequence designated SEQ. I.D. No. 3, comprising: (a) obtaining the peptide in a purified form; (b) immunizing an organism capable of producing antibodies against the purified peptide; (c) collecting the produced antibodies; (d) combining the produced antibodies and the purified peptide under conditions to form a complex; and (e) determining which produced antibodies form a complex with the purified peptide so as to obtain antibodies to the peptide.

One could perform the above-described well-known method in rabbits or mice.

This invention also provides the antibodies obtained from the above-described methods, specifically the monoclonal antibodies. Further, the invention provides antibodies capable of detecting a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and incapable of detecting the major surface antigen of a wild type hepatitis B virus, as well as, antibodies capable of detecting a peptide, wherein the peptide has an amino acid sequence comprising amino acid residues 298 through 320 of the amino acid sequence designated SEQ. I.D. No. 3.

This invention also provides use of an isolated nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino

acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine for determining whether a subject is infected with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine), wherein such determination comprises: (a) obtaining an appropriate nucleic acid sample from the subject; and (b) determining whether the nucleic acid sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine.

A means of determination is where the nucleic acid sample in step (a) comprises mRNA corresponding to the transcript of DNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and wherein the determining of step (b) comprises: (i) contacting the mRNA with the above-described oligonucleotide under conditions permitting binding of the mRNA to the oligonucleotide so as to form a complex; (ii) isolating the complex so formed; and (iii) identifying the mRNA in the isolated complex so as to thereby determine whether the mRNA is, or is derived from, a nucleic acid which encodes the polypeptide.

Another example is where the nucleic acid sample in step (a) comprises mRNA corresponding to the transcript of DNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide

having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and wherein the determining of step (b) comprises: (i) translating the mRNA under suitable conditions to obtain an amino acid sequence; and (ii) comparing the amino acid sequence of step (i) with the amino acid sequence of an isolated nucleic acid which encodes a polypeptide, wherein the polypeptide has an amino acid sequence substantially identical to amino acid residues 174 through 400 of the amino acid sequence designated SEQ. I.D. No. 3 so as to thereby determine whether the nucleic acid sample is, or is derived from, a nucleic acid which encodes the polypeptide. A further example is where the determining of step (b) comprises: (i) amplifying the nucleic acid present in the sample of step (a); and (ii) detecting the presence of polypeptide in the resulting amplified nucleic acid.

This invention provides the use of an antibody that recognizes a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus for determining whether the subject has a predisposition for hepatocellular carcinoma, wherein such determination comprises: (a) obtaining an appropriate nucleic acid sample from the subject; and (b) determining whether the nucleic acid sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, by contacting the sample under appropriate conditions to bind to the antibodies of claim 35 so as to determine whether the subject has a predisposition for hepatocellular carcinoma.

This invention also provides use of antibodies capable of

detecting a polypeptide which is a mutant major surface antigen of a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) for determining whether a subject is
5 infected with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine), wherein such determination comprises: (a) obtaining an appropriate sample from the subject; and (b) determining whether the sample from step (a) is, or is derived
10 from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position
15 number 145 of such polypeptide is an arginine, rather than a glycine by contacting the sample under appropriate conditions to bind to the antibodies so as to determine whether a subject is infected. Furthermore, the antibody may also be capable detecting a peptide, wherein the peptide has an amino acid
20 sequence comprising amino acid residues 298-320 of the amino acid sequence designated SEQ. I.D. No. 3.

In the above-described uses, the isolated nucleic acid, oligonucleotide or antibody may be labeled with a detectable
25 marker. Examples of detectable markers include radioactive isotopes, fluorophors and enzymes.

In a specific embodiment, the sample includes, but is not limited to, blood, tissue or sera.

30 This invention also provides a method for identifying a chemical compound for the manufacture of a medicament which is capable of treating infection by a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145
35 Singapore Strain (Glycine to Arginine) which comprises: (a) contacting a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino

acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, with the chemical compound under conditions permitting binding
5 between the polypeptide and the chemical compound; (b) detecting specific binding of the chemical compound to the polypeptide; and (c) determining whether the chemical compound inhibits the polypeptide so as to identify a chemical compound which is capable of treating infection by the viral strain.

10

This invention also provides a method for identifying a chemical compound for the manufacture of a medicament which is capable of preventing infection by a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-
15 145 Singapore Strain (Glycine to Arginine), which comprises: (a) contacting a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type
20 hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, with the chemical compound under conditions permitting binding between the polypeptide and the chemical compound; (b)
25 detecting specific binding of the chemical compound to the polypeptide; and (c) determining whether the chemical compound inhibits the polypeptide so as to identify a chemical compound which is capable of preventing infection by the viral strain.

This invention further provides a composition comprising a
30 polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is
35 an arginine, rather than a glycine, or derivative thereof, the amounts of such polypeptide being effective to stimulate or enhance antibody production in a subject, and a pharmaceutically acceptable carrier.

The actual effective amount will be based upon the size of the polypeptide, the biodegradability of the polypeptide, the bioactivity of the polypeptide and the bioavailability of the polypeptide. If the polypeptide does not degrade quickly, is
5 bioavailable and highly active, a smaller amount will be required to be effective. The effective amount will be known to one of skill in the art; it will also be dependent upon the form of the polypeptide, the size of the polypeptide and the bioactivity of the polypeptide. Use of an adjuvant for
10 example, would lower the required amount of the polypeptide. One of skill in the art could routinely perform empirical activity tests to determine the bioactivity in bioassays and thus determine the effective amount.

15 Pharmaceutically acceptable carriers are well known to those skilled in the art and include, but are not limited to, 0.01-0.1M and preferably 0.05M phosphate buffer or 0.8% saline. Additionally, such pharmaceutically acceptable carriers may be aqueous or non-aqueous solutions, suspensions, and emulsions.
20 Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media.
25 Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers such as those based on Ringer's dextrose, and the like. Preservatives and other
30 additives may also be present, such as, for example, antimicrobials, antioxidants, chelating agents, inert gases and the like.

This invention further provides a composition comprising a
35 peptide, wherein the peptide has an amino acid sequence comprising amino acid residues 298 through 320 of the amino acid sequence designated SEQ. I.D. No. 3 or derivative thereof, the amounts of such peptide being effective to

stimulate or enhance antibody production in a subject, and a pharmaceutically acceptable carrier.

5 This invention further provides compositions comprising the chemical compound identified by the above-described methods in an amount effective to treat or prevent infection by a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) and a pharmaceutically effective carrier.

10 This invention provides the use of the above-described compositions as medicaments for treating and/or preventing hepatocellular carcinoma.

15 This invention also provides use of the above-identified compositions for treating a subject infected with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine).

20 This invention also provides use of the above-identified compositions for preventing infection by a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) in a subject which comprises administering an effective amount.

25 This invention further provides a method of screening tissues and bodily fluids from a subject for a strain of hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) which comprises:

30 (a) obtaining an appropriate sample of bodily fluid from the subject; (b) determining the presence of a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface

35 antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine in the sample of step (a) so as to screen the sample for the strain. In embodiments of

this method, the bodily fluid comprises blood, sera, or a nucleic acid sample of blood or sera.

This invention provides a method for determining whether a
5 subject has a predisposition for hepatocellular carcinoma,
which comprises: (a) obtaining an appropriate nucleic acid
sample from the subject; and (b) determining whether the
nucleic acid sample from step (a) is, or is derived from, a
nucleic acid encoding a polypeptide which is a mutant major
10 surface antigen of a strain of hepatitis B virus, such
polypeptide having an amino acid sequence which differs from
the amino acid sequence of a major surface antigen of a wild
type hepatitis B virus in that the amino acid at position
number 145 of such polypeptide is an arginine, rather than a
15 glycine, thereby determining whether the subject has a
predisposition for hepatocellular carcinoma.

This invention also provides the above-described method,
wherein the nucleic acid sample in step (a) comprises mRNA
20 encoding a polypeptide which is a mutant major surface antigen
of a strain of hepatitis B virus, such polypeptide having an
amino acid sequence which differs from the amino acid sequence
of a major surface antigen of a wild type hepatitis B virus in
that the amino acid at position number 145 of such polypeptide
25 is an arginine, rather than a glycine, and wherein the
determining of step (b) comprises: (i) contacting the mRNA
with the above-described oligonucleotides under conditions
permitting binding of the mRNA to the oligonucleotide so as to
form a complex; (ii) isolating the complex so formed; and
30 (iii) identifying the mRNA in the isolated complex so as to
thereby determine whether the mRNA is, or is derived from, a
nucleic acid which encodes the polypeptide.

This invention further provides the above-described method,
35 wherein the nucleic acid sample in step (a) comprises mRNA
encoding a polypeptide which is a mutant major surface antigen
of a strain of hepatitis B virus, such polypeptide having an
amino acid sequence which differs from the amino acid sequence

of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and wherein the determining of step (b) comprises: (i) translating the mRNA
5 under suitable conditions to obtain an amino acid sequence; and (ii) comparing the amino acid sequence of step (i) with the amino acid sequence encoded by the isolated nucleic acid described above so as to determine whether the nucleic acid sample is, or is derived from, a nucleic acid which encodes
10 the polypeptide.

This invention also provides the above-described method, wherein the determining of step (b) comprises: (i) amplifying the nucleic acid present in the sample of step (a); and (ii)
15 detecting the presence of polypeptide in the resulting amplified nucleic acid.

This invention further provides the above-described method for determining whether a subject has a predisposition for
20 hepatocellular carcinoma, which comprises: (a) obtaining an appropriate sample from the subject; and (b) determining whether the sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such
25 polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, by contacting the sample under appropriate conditions
30 to bind to the above-described antibodies so as to determine whether the subject has a predisposition for hepatocellular carcinoma.

This invention provides the above-described methods, wherein
35 the oligonucleotide or antibody is labeled with a detectable marker.

This invention also provides the above-described methods,

wherein the detectable marker is a radioactive isotope, a fluorophor or an enzyme.

This invention also provides the above-described methods,
5 wherein the sample comprises blood, tissue or sera.

This invention further provides a method for identifying a chemical compound for the manufacture of a medicament which is capable of treating hepatocellular carcinoma which comprises:
10 (a) contacting a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number
15 145 of such polypeptide is an arginine, rather than a glycine, with the chemical compound under conditions permitting binding between the polypeptide and the chemical compound; (b) detecting specific binding of the chemical compound to the polypeptide; and (c) determining whether the chemical compound
20 binds to the polypeptide so as to identify a chemical compound which is capable of treating hepatocellular carcinoma.

This invention provides a method for identifying a chemical compound for the manufacture of a medicament which is capable
25 of preventing hepatocellular carcinoma, which comprises: (a) contacting a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type
30 hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, with the chemical compound under conditions permitting binding between the polypeptide and the chemical compound; (b)
35 detecting specific binding of the chemical compound to the polypeptide; and (c) determining whether the chemical compound binds to the polypeptide so as to identify a chemical compound which is capable of preventing hepatocellular carcinoma.

Additionally, this invention provides a composition comprising the chemical compound identified by the above-described methods in an amount effective to treat hepatocellular carcinoma and a pharmaceutically effective carrier.

5

This invention also provides a composition comprising the chemical compound identified by the above-described methods in an amount effective to prevent hepatocellular carcinoma and a pharmaceutically effective carrier.

10

This invention further provides a method treating a subject with hepatocellular carcinoma which comprises administering an effective amount of the above-described compositions.

15 This invention further provides a method preventing hepatocellular carcinoma in a subject which comprises administering an effective amount of the above-described compositions.

20 This invention also provides a hepatitis vaccine, comprising a mutant form of the surface antigen of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of the major surface antigen of hepatitis B in that the amino acid at position number 145 of
25 such polypeptide is an arginine rather than a glycine.

This invention also provides the above-described vaccine and an adjuvant.

30 This invention is illustrated in the Experimental Details section which follows. These sections are set forth to aid in an understanding of the invention but are not intended to, and should not be construed to, limit in any way the invention as set forth in the claims which follow thereafter.

35

Experimental Details

In the method described below, the hepatitis B virus carrying

the mutation at amino acid residue 145 of the major surface antigen was isolated, and its nucleotide sequence was determined.

5 Serum sample (20/815c) was obtained from an 11-year old child born to a mother carrying the wild type surface antigen. The child tested surface antigen negative at birth and was subjected to a combined treatment of hepatitis B immunoglobulin (HBIG) and plasma-derived vaccine. He was
10 tested surface antigen positive one year later and carried an Arginine mutation at amino acid residue 145 in the major surface antigen. The viral DNA was extracted prior to the determination of its sequence in the present invention.

15 As described in the Examples below, the genome of this hepatitis B mutant virus carrying a mutation at amino acid residue 145 of the major surface antigen consists of 3215 nucleotides which are identical to those of the wild type virus of the same subtype (adw). Open reading frames (ORFs)
20 coding for the major viral proteins are found at corresponding positions when compared to the wild type virus. Position 1 in the mutant hepatitis B virus genome is defined according to that in the wild type virus, corresponding to the restriction site EcoRI which is absent in the hepatitis B virus in the
25 present invention due to changes in the nucleotide sequence.

The structure of the different ORFs in the mutant virus genome reported are summarized in Figure 1 and their locations are indicated as follows:

- 30 - DNA polymerase gene starts at position 2307 and ends at position 1623, therefore consisting of 2532 nucleotides and coding for 843 amino acid residues;
- Large surface antigen gene starts at position 2848 and ends at position 835, therefore consisting of 1203 nucleotides and
35 coding for 400 amino acid residues. This large surface antigen overlaps the middle surface antigen starting at position 3205 and the major surface antigen which starts at position 155. Both the middle (consisting of 281 amino acids

residues) and the major (consisting of 226 amino acid residues) surface antigens end at the same position as the large surface antigen;

- Core gene starts at position 1814 and ends at position 2452, therefore consisting of 639 nucleotides and coding for 212 amino acid residues; and

- Trans-activating X gene starts at position 1374 and ends at position 1838, therefore consisting of 465 nucleotides and coding for 154 amino acid residues.

10

Furthermore, sequence analysis has established this mutant hepatitis B virus as belonging to adw subtype, as indicated by a lysine residue at both positions 122 and 160 in the major surface antigen. Consistent with previous analysis of the "a" epitope by direct sequencing, the vaccine-induced mutation (from Glycine to Arginine) is found at amino acid residue 145 of the major surface antigen.

20 Compared with the wild type hepatitis B virus deposited in the Genbank database (accession number D00329), the identity of this hepatitis B viral strain is at 89.4% for the nucleotide sequence. The identity of different viral proteins of the present mutant hepatitis B virus as compared with its wild type counterparts is at 88.3%, 87.7%, 93.4% and 87% for DNA polymerase (PIR - Protein Identification Resources accession number P93460), large surface antigen (PIR accession number A93460), core (PIR accession number C93460) and trans-activating X (PIR accession number A31289) proteins, respectively.

30

The hepatitis B virus genome in the present invention carrying a vaccine-induced mutation at amino acid residue 145 (Glycine to Arginine) of the major surface antigen, can be used as material to design oligonucleotides specific to the mutant virus genome. These oligonucleotides can be used as material for highly specific diagnostic agents that detect virus carrying a vaccine-induced mutation at amino acid residue 145 of the major surface antigen.

The hepatitis B virus genome in the present invention, with a vaccine-induced mutation at amino acid residue 145 (Glycine to Arginine) of the major surface antigen, can be used as material to produce proteins of the invention by expressing a vector that carries the relevant coding region, and which can replicate in a host cell such as Escherichia coli, by standard DNA recombinant technology.

Proteins of the present invention are useful as material for highly specific diagnostic agents capable of detecting hepatitis B virus carrying a vaccine-induced mutation at amino acid residue 145 of the major surface antigen. Using known methods, these same proteins can be used to produce polyclonal and monoclonal antibodies.

Polyclonal and monoclonal antibodies can be used as material for diagnostic agents to detect with high specificity antigens of hepatitis B virus, with a vaccine-induced mutation at amino acid residue 145 (Glycine to Arginine) of the major surface antigen.

A detection system using each protein of the present invention or proteins with partial replacement of amino acids, and a detection system using monoclonal or polyclonal antibodies to such proteins, are useful as highly specific diagnostic agents for hepatitis B virus with vaccine-induced mutation at amino acid residue 145 of the major surface antigen, and are effective to detect and screen out such virus from transfusion bloods or blood derivatives. The proteins or antibodies to such proteins, can be used as a material for development of prophylactic and therapeutic vaccine against such virus.

It is well known that one or more nucleotides in a DNA sequence can be substituted by other nucleotides to produce the same protein. The present invention also concerns such nucleotide changes which code for proteins reported in this invention. It is equally well known that one or more amino acids in a protein sequence can be replaced by other analogous

amino acids, as defined by their hydrophilic properties or charges, to produce an analog of the amino acid sequence. Any analogs of the proteins of the present invention involving amino acid replacement, deletions, or by isosteres (modified amino acids that bear close structural and spatial similarity to protein amino acids), amino acid addition, or isostere addition can be utilized, provided that the resulting sequences elicit antibodies recognizing hepatitis B virus with a vaccine-induced mutation at amino acid mutation 145 (Glycine to Arginine) of the major surface antigen.

EXAMPLES

The nucleotide sequence and the deduced amino acid sequence of hepatitis B virus, carrying a vaccine-induced mutation at amino acid residue 145 (Glycine to Arginine) of the major surface antigen, were determined in the following manner:

1. Isolation of Viral DNA

The viral DNA was isolated from a serum sample (20/815c) obtained from the serum of an 11-year old Chinese child who was born to a mother of wild type hepatitis B virus surface antigen. Despite negative test results at birth, he was given combined treatment of HBIG and the plasma-derived vaccine and tested positive for hepatitis B virus surface antigen one year later. Sequence analysis of the "a" epitope indicated the presence of a vaccine-induced mutation at amino acid residue 145 of the major surface antigen.

30

The isolation method used was:

200 μ l of the serum sample was added with 400 μ l of the lysis buffer (Tris chloride 10 mM, pH7.4, EDTA 1 mM, and sodium dodecyl sulfate 2%) and 25 μ l of proteinase K (20 mg/ml), incubated at 65 C for 3 hours. Viral DNA was then extracted by phenol/chloroform and precipitated by ethanol.

2. Amplification of Viral DNA by Polymerase Chain Reaction

(PCR)

- The virus genome was amplified by polymerase chain reaction (PCR) using 3 sets of overlapping oligonucleotides, which were designed according to the wild type hepatitis B virus of adw subtype. Various restriction enzyme sites were included to facilitate the cloning of the PCR products. The position of these oligonucleotides is shown in Fig. 2 and indicated as follows:
- 10 - Flag 1 (ATAAGCTTATGCCCCTATCTTATCAACACTTCCGGA) (SEQ. I.D. No. 6) starts at the initiation site of the coding region of DNA polymerase, at position 2307 of the viral nucleotide sequence and matches the coding strand (sense oligonucleotide). An additional HindIII restriction enzyme site is underlined;
 - 15 - Xba3 (GAGTCTAGACTCTGCGGTATTGTGA) (SEQ. I.D. No. 7) starts at the internal restriction enzyme site XbaI, at position 250 of the viral nucleotide sequence and matches the complementary strand (anti-sense oligonucleotide). An additional XbaI restriction enzyme site is underlined;
 - 20 - Xba5 (GAGTCTAGACTCGTGGTGGACTTCT) (SEQ. I.D. No. 8) starts at the internal XbaI site, at the same location as that of Xba3 oligonucleotide but matches the coding strand (sense oligonucleotide). An additional XbaI restriction enzyme site is underlined;
 - 25 - Common 3 (TGAGAATTCTCACGGTGGTCTCCATGCGACGT) (SEQ. I.D. No. 9) starts at the stop codon of the DNA polymerase, at position 1623 of the viral nucleotide sequence and matches the complementary strand (anti-sense oligonucleotide). An additional EcoRI restriction enzyme site is underlined;
 - 30 - V11 (TTTGTTTACGTCCCGT) (SEQ. I.D. No. 10) starts near the initiation site of the X gene, at position 1420 of the viral nucleotide sequence and matches the coding strand (sense oligonucleotide);
 - HindIIIADW3 (CTAAGCTTAGTTTCCGGAAGTGTGAT) (SEQ. I.D. No. 11)
 - 35 starts close to the initiation site of the DNA polymerase, at position 2340 and matches the complementary strand (anti-sense oligonucleotide). An additional Hind III restriction enzyme site is underlined.

Using viral DNA as a template, PCR was then carried out on a DNA Thermal Cycler (Perkin-Elmer. Cetus) for 35 cycles using Pfu polymerase (Stratagene, U.S.A.), each cycle consisting of 1.5 minutes at a denaturing temperature of 94 °C, 2 minutes at an annealing temperature of 53 °C and 4 minutes at an extension temperature of 72 °C. The following combinations of oligonucleotides were used: Flag1/Xba3, Xba5/Common3 and V11/HindIIIADW3, and generating amplification products of 1.2 kb, 1.4 kb and 1.1 kb, respectively.

3. Cloning of the Amplified Viral DNA Fragments.

Amplified viral DNA fragment from Flag1/Xba3 (1.2 kb) was subjected to restriction enzyme digestion by HindIII and XbaI prior to cloning in a BlueScript plasmid pre-treated by the same restriction enzymes. Similar digestion with XbaI and EcoRI was applied to PCR product from Xba5/Common3 (1.4 kb) prior to cloning in a BlueScript plasmid pre-treated by XbaI and EcoRI. On the other hand, the DNA fragment amplified with V11 and HindIIIADW3 (1.1 kb) was directly cloned into ZeroBlunt plasmid, developed by InvitroGen (U.S.A.) for cloning blunt-end DNA fragments.

4. Determination of Nucleotide Sequence

Nucleotide sequence of the vaccine-induced hepatitis B virus in the present invention was determined on plasmid DNA template by chain-terminating inhibitors, using the Sequenase DNA Sequencing Kit (United States Biochemical Corp.). To facilitate the sequencing procedure, various internal oligonucleotides were designed (from V1 to V13) according to the wild type hepatitis B virus, and their positions are indicated in Fig. 2.

From the analysis described above, the full-length nucleotide sequence of the hepatitis B virus carrying a vaccine-induced mutation at amino acid residue 145 (Glycine to Arginine) of the major surface antigen was determined as shown in Figure 3.

The deduced amino acid sequences coding for the major viral proteins are shown in Figures 4-7: hepatitis B viral DNA polymerase (Figure 4), the large surface antigen (Figure 5), the core protein (Figure 6) and the trans-activating X protein
5 (Figure 7).

Alignment of the virus sequence in the present invention with other hepatitis B viral sequences, available in the Genbank database, will point to specific sequence differences which in
10 turn can be used to design DNA probes. A detection system using polymerase chain reaction (PCR) can then be developed. Such PCR reactions will involve combinations of oligonucleotides specific to hepatitis B virus with a vaccine-induced mutation at the amino acid residue 145
15 (Glycine to Arginine) of the major surface antigen, thereby allowing highly specific detection of these mutant viral DNAs. Briefly, viral DNA can be extracted as described in this invention. PCR reactions can be performed using specific oligonucleotides using similar cycling conditions described
20 above. Results can then be analyzed after resolving PCR products on a 1 % agarose gel.

According to known immunological procedures, it is possible to determine epitopes from protein sequences such as those in
25 Figures 4-7. Determination of these epitopes specific to hepatitis B virus with vaccine-induced mutation at amino acid residue 145 of the major surface antigen will allow the synthesis of peptides using genetic engineering methods, synthesis of the proteins, production of the antibodies,
30 development of specific diagnostic reagents, development of prophylactic and therapeutic vaccines, and antiviral agents.

A detection system for antibodies against hepatitis B virus, with a vaccine-induced mutation at amino acid residue 145 of
35 the major surface antigen, can be developed using polyvinyl microtiter plates and the sandwich method. Briefly, 50 μ l of 5 μ g/ml concentration of a hepatitis B virus (vaccine-induced mutant) peptide can be dispensed in each well of the

microtiter plates and incubated overnight at room temperature for consolidation. Similar procedures can be applied to 's' protein purified from host cells such as Escherichia coli. The microplate wells can be washed five times with physiological saline solution containing 0.05% Tween 20. For overcoating, 100 μ l of NaCl buffer containing 30% (v/v) of calf serum and 0.05% Tween 20 (CS buffer) can be dispensed in each well and discarded after incubation for 30 minutes at room temperature.

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To determine antibodies specific for the vaccine-induced mutant hepatitis B virus antibodies in serum, the primary reaction can be carried out such that 50 μ l of the CS buffer and 10 μ l of a serum sample can be dispensed in each microplate well and incubated on a microplate vibrator for one hour at room temperature. After completion of the primary reaction, microplate wells are washed five times as described above.

20 In the secondary reaction, 1 ng of horseradish peroxidase labeled anti-human IgG mouse monoclonal antibodies dissolved in 50 μ l of calf serum can be dispensed in each microplate well, and incubated on a microplate vibrator for one hour at room temperature. Upon completion, wells can be washed five times in the same way. After addition of hydrogen peroxide (as substrate) and 50 μ l of O-phenylendiamine solution (as color developer) in each well, and after incubation for 30 minutes at room temperature, 50 μ l of 4M sulphuric acid solution can be dispensed in each well to stop further color development and for reading absorbance at 490nm.

The present invention makes possible detection of vaccine-induced mutant hepatitis B virus, in particular those carrying a mutation at amino acid residue 145 of the major surface antigen. Such mutant hepatitis B virus have hitherto escaped the detection using conventional antibody-based methods, and the present invention also provides detection systems capable of highly specific and sensitive detection at

an early stage of infection.

In addition, these features allow accurate diagnosis of patients at an early stage of the disease and also help to
5 remove with higher efficiency blood contaminated with vaccine-induced mutant hepatitis B virus through using a screening test of donor bloods.

Proteins and their antibodies under the present invention can
10 be utilized for development of prophylactic and therapeutic vaccines, as well as, immunological pharmaceuticals. Sequence information on structural genes of these mutant viruses will be helpful in developing detection systems of the relevant protein antigens and antibodies.

15 Antigen-antibody complexes can be detected by known methods. Specific monoclonal and polyclonal antibodies can be raised by immunizing animals such as mice and rabbits with peptides or proteins specific to mutant vaccine-induced hepatitis B
20 viruses. Inhibitory antiviral agents can be designed and targeted against these proteins and molecules in cell culture or in vivo.

The present invention is based on studies on an isolated virus
25 genome with a vaccine-induced mutation at amino acid residue 145 (Glycine to Arginine) of the major surface antigen. The invention makes possible highly specific detection of these vaccine-induced mutant hepatitis B virus and provides material such as protein, polyclonal and monoclonal antibodies for
30 development of such detection system.

We have evidence showing that when expressed in a mammalian expression system, the major surface antigen (HBsAg) of the mutant HBV reported in our invention is detected as a 22kDa
35 protein on a Coomassie-blue stained SDS-PAGE gel, whereas the wild type HBsAg is detected as a 25kDa protein. Since the only glycosylation site on the HBsAg is located in close proximity (Asparagine at position 146) to the mutation at

position 145, it is very likely that the drastic change from Glycine to Arginine at position 145 affects the glycosylation process in the mutant HBsAg. This defective glycosylation process would in turn result in the smaller protein as
5 observed in our studies. As the antigenicity of a protein is adversely affected by the extent of its glycosylation, we predict that the mutant HBsAg reported in our invention is more antigenic than the wild type HBsAg. This prediction is further supported by the structural analysis using the method
10 developed by Hopp and Woods.

Our serological studies also indicate major differences between wild type HBV carriers and those carrying the mutant HBV reported in our invention, one such difference is that the
15 serum viral DNA load is significantly lower at around 5pg per ml as measured by an ¹²⁵I hybridization assay (Abbott Laboratories, U.S.A.). In marked contrast, the wild type HBV DNA load is much higher at over 100pg per ml. In addition, the anti-Hepatitis B surface antigen (anti-HBs) level is
20 detectable at 10 IU per ml in the mutant HBV reported in our invention, whereas such antibodies remained undetectable in carriers of wild type HBV.

In the process of sequence determination, we have observed
25 that a few oligonucleotides (each of them longer than 17 bases) designed according to the wild type HBV sequence, failed to hybridize to the mutant viral DNA reported in our invention. Two such oligonucleotides are located in the genome at positions 2711 to 2729 and 2902 to 2920. Our
30 results, therefore, point to the structural differences between the wild type and mutant HBV genomes.

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What is claimed is:

1. An isolated strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) which constituent viral genome is deposited under Accession Nos. P97121504, P97121505 and P97121506 with the European Collection of Cell Culture on 15th December 1997.
2. An isolated nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine.
3. The isolated nucleic acid of claim 2, wherein the polypeptide is being encoded by nucleotides 155 through 835 of the nucleic acid sequence designated SEQ. I.D. No. 1.
4. The isolated nucleic acid of claim 3, comprising nucleotides "AGA" in positions 587-589.
5. The isolated nucleic acid of claim 2, wherein the nucleic acid is DNA.
6. The isolated nucleic acid of claim 2, wherein the nucleic acid is RNA.
7. The isolated nucleic acid of claim 5, wherein the nucleic acid is cDNA.
8. The isolated nucleic acid of claim 5, wherein the nucleic acid is genomic DNA.

9. The isolated nucleic acid of claim 2, wherein the polypeptide has an amino acid sequence substantially the same as amino acid residues 174 through 400 of the amino acid sequence designated SEQ. I.D. No. 3.
- 5 10. An isolated nucleic acid which encodes a peptide, wherein the peptide is encoded by a nucleic acid molecule comprising nucleotides 527 through 595 of SEQ. I.D. No. 1.
- 10 11. An isolated nucleic acid which encodes a peptide, wherein the peptide has an amino acid sequence comprising amino acid residues 298 through 320 of the amino acid sequence designated SEQ. I.D. No. 3.
- 15 12. A vector comprising an isolated nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine and operatively linked to a promoter of RNA transcription.
- 20 13. A vector comprising an isolated nucleic acid encoding a peptide, wherein the peptide is encoded by a nucleic acid molecule comprising nucleotides 527 through 595 of SEQ. I.D. No. 1.
- 25 14. The vector of claim 12 or 13, wherein the vector comprises viral DNA.
- 30 15. A host vector system for the production of a polypeptide which comprises the vector of claim 12 in a suitable host.
- 35 16. A host vector system for the production of a peptide

which comprises the vector of claim 13 in a suitable host.

- 5 17. A method of producing a polypeptide which comprises growing the host vector system of claim 15 under suitable conditions permitting production of the polypeptide and recovering the polypeptide so produced.
- 10 18. A method of producing a peptide which comprises growing the host vector system of claim 16 under suitable conditions permitting production of the polypeptide and recovering the polypeptide so produced.
- 15 19. A method of obtaining a polypeptide in purified form which comprises:
 (a) introducing the vector of claim 12 into a suitable host cell;
 (b) culturing the resulting host cell so as to produce the polypeptide;
20 (c) recovering the polypeptide produced in step (b);
 and
 (d) purifying the polypeptide so recovered.
- 25 20. A method of obtaining a polypeptide in purified form which comprises:
 (a) introducing the vector of claim 13 into a suitable host cell;
 (b) culturing the resulting host cell so as to produce the polypeptide;
30 (c) recovering the polypeptide produced in step (b);
 and
 (d) purifying the polypeptide so recovered.
- 35 21. A purified polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid

at position number 145 of such polypeptide is an arginine rather than a glycine.

22. A purified polypeptide obtained from the method of claim 19.
23. A purified peptide, wherein the peptide has an amino acid sequence comprising amino acid residues 298 through 320 of the amino acid sequence designated SEQ. I.D. No. 3.
24. A purified peptide obtained from the method of claim 20.
25. An oligonucleotide of at least 15 nucleotides capable of specifically hybridizing with a unique sequence of nucleotides within a nucleic acid which encodes a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine, without hybridizing to any sequence of nucleotides within a nucleic acid which encodes the major surface antigen of a wild type hepatitis B virus.
26. The oligonucleotide of claim 25 comprising nucleotides 527 through 595 of SEQ. I.D. No. 1.
27. A method of obtaining antibodies to a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and not to the major surface antigen of a wild type hepatitis B virus, comprising:

- (a) obtaining the polypeptide in a purified form;
 - (b) immunizing an organism capable of producing antibodies against the purified polypeptide;
 - (c) collecting the produced antibodies;
 - 5 (d) combining the produced antibodies and the purified polypeptide under conditions to form a complex; and
 - (e) determining which produced antibodies form a complex with the purified polypeptide so as to obtain antibodies to the polypeptide.
- 10 28. The method of claim 27, wherein the polypeptide is being encoded by nucleotides 155 through 835 of the nucleic acid sequence designated SEQ. I.D. No. 1.
- 15 29. The method of claim 27, wherein the polypeptide has an amino acid sequence substantially the same as amino acid residues 174 through 400 of the amino acid sequence designated SEQ. I.D. No. 3.
- 20 30. The method of claim 27, wherein the organism comprises a rabbit or a mouse.
31. A method of obtaining antibodies to a peptide, wherein the peptide has an amino acid sequence comprising amino acid residues 298 through 320 of the amino acid sequence designated SEQ. I.D. No. 3, comprising:
- 25 (a) obtaining the peptide in a purified form;
- (b) immunizing an organism capable of producing antibodies against the purified peptide;
- 30 (c) collecting the produced antibodies;
- (d) combining the produced antibodies and the purified peptide under conditions to form a complex; and
- (e) determining which produced antibodies form a complex with the purified peptide so as to obtain antibodies to the peptide.
- 35 32. The method of claim 31, wherein the organism comprises a rabbit or a mouse.

33. The antibodies obtained in claim 27 or 31.
34. Monoclonal antibodies of the antibodies of claim 33.
- 5 35. Antibodies capable of detecting a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that
10 the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and incapable of detecting the major surface antigen of a wild type hepatitis B virus.
- 15 36. Antibodies capable of detecting a peptide, wherein the peptide has an amino acid sequence comprising amino acid residues 298 through 320 of the amino acid sequence designated SEQ. I.D. No. 3.
- 20 37. Use of a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that
25 the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine for determining whether a subject is infected with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine),
30 wherein such determination comprises
(a) obtaining an appropriate nucleic acid sample from the subject; and
(b) determining whether the nucleic acid sample from
35 step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a

major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine.

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38. The use of claim 37, wherein the nucleic acid sample in step (a) comprises mRNA corresponding to the transcript of DNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and wherein the determining of step (b) comprises:

15

- (i) contacting the mRNA with the oligonucleotide of claim 25 under conditions permitting binding of the mRNA to the oligonucleotide so as to form a complex;
- (ii) isolating the complex so formed; and
- (iii) identifying the mRNA in the isolated complex so as to thereby determine whether the mRNA is, or is derived from, a nucleic acid which encodes the polypeptide.

25

39. The use of claim 37, wherein the nucleic acid sample in step (a) comprises mRNA corresponding to the transcript of DNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and wherein the determining of step (b) comprises:

35

- (i) translating the mRNA under suitable conditions to obtain an amino acid sequence; and
- (ii) comparing the amino acid sequence of step (i)

with the amino acid sequence encoded by the isolated nucleic acid of claim 9 so as to determine whether the nucleic acid sample is, or is derived from, a nucleic acid which encodes the polypeptide.

5

40. The use of claim 37, wherein the determining of step (b) comprises:

- 10 (i) amplifying the nucleic acid present in the sample of step (a); and
(ii) detecting the presence of polypeptide in the resulting amplified nucleic acid.

15 41. Use of antibodies capable of detecting a polypeptide which is a mutant major surface antigen of a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) for determining whether a subject is infected with a strain of Hepatitis B virus designated Human
20 Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine), wherein such determination comprises:

- (a) obtaining an appropriate sample from the subject;
and
25 (b) determining whether the sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from
30 the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, by contacting the sample under appropriate conditions to bind to
35 the antibodies of claim 35 or 36 so as to determine whether a subject is infected.

42. The use of claim 37, 38 or 41, wherein the isolated

nucleic acid, oligonucleotide, or antibody is labeled with a detectable marker.

43. The use the claim 42, wherein the detectable marker is a radioactive isotope, a fluorophor, or an enzyme.
44. The use of claim 37, wherein the sample comprises blood, tissue, or sera.
45. A method for identifying a chemical compound for the manufacture of a medicament which is capable of treating infection by a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine), which comprises:
- (a) contacting a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, with the chemical compound under conditions permitting binding between the polypeptide and the chemical compound;
 - (b) detecting specific binding of the chemical compound to the polypeptide; and
 - (c) determining whether the chemical compound binds to the polypeptide so as to identify a chemical compound which is capable of treating infection by the viral strain.
46. A method for identifying a chemical compound for the manufacture of a medicament which is capable of preventing infection by a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine), which comprises:
- (a) contacting a polypeptide which is a mutant major

5 surface antigen of a strain of hepatitis B virus,
such polypeptide having an amino acid sequence
which differs from the amino acid sequence of a
major surface antigen of a wild type hepatitis B
virus in that the amino acid at position number 145
of such polypeptide is an arginine, rather than a
glycine, with the chemical compound under
conditions permitting binding between the
polypeptide and the chemical compound;

10 (b) detecting specific binding of the chemical compound
to the polypeptide; and

(c) determining whether the chemical compound binds to
the polypeptide so as to identify a chemical
compound which is capable of preventing infection
15 by the viral strain.

47. A composition comprising a polypeptide which is a mutant
major surface antigen of a strain of hepatitis B virus,
such polypeptide having an amino acid sequence which
20 differs from the amino acid sequence of a major surface
antigen of a wild type hepatitis B virus in that the
amino acid at position number 145 of such polypeptide is
an arginine, rather than a glycine, or derivative
thereof, the amounts of such polypeptide being effective
25 to stimulate or enhance antibody production in a subject,
and a pharmaceutically acceptable carrier.

48. A composition comprising a peptide, wherein the peptide
has an amino acid sequence comprising amino acid residues
30 298 through 320 of the amino acid sequence designated
SEQ. I.D. No. 3. or derivative thereof, the amounts of
such peptide being effective to stimulate or enhance
antibody production in a subject, and a pharmaceutically
acceptable carrier.

35 49. A composition comprising the chemical compound identified
by the method of claim 45 in an amount effective to treat
infection by a strain of Hepatitis B virus designated

Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) and a pharmaceutically effective carrier.

- 5 50. A composition comprising the chemical compound identified by the method of claim 46 in an amount effective to prevent infection by a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) and a
10 pharmaceutically effective carrier.
51. Use of the composition of claim 47 or 48 for treating a subject infected with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-
15 145 Singapore Strain (Glycine to Arginine).
52. Use of the composition of claim 45 for treating a subject infected with a strain of hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore
20 Strain (Glycine to Arginine).
53. Use of the composition of claim 47 or 48 for preventing infection by a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore
25 Strain (Glycine to Arginine) in a subject.
54. Use of the composition of claim 46 for preventing infection with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore
30 Strain (Glycine to Arginine) in a subject.
55. A method of screening bodily fluids from a subject for a strain of hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine
35 to Arginine) which comprises:
- (a) obtaining an appropriate sample of bodily fluid from the subject;
 - (b) determining the presence of a polypeptide which is

5 a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, in the sample of step (a) so as to screen the sample for the strain.

10 56. The method of claim 55, wherein the bodily fluid comprises blood, sera, or a nucleic acid sample of blood or sera.

15 57. Use of an antibody that recognizes a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus for determining whether the subject has a predisposition for hepatocellular carcinoma, wherein such determination comprises:

20 (a) obtaining an appropriate nucleic acid sample from the subject; and

(b) determining whether the nucleic acid sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, by contacting the sample under appropriate conditions to bind to the antibodies of claim 35 so as to determine whether the subject has a predisposition for hepatocellular carcinoma.

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35 58. The method of claim 57, wherein the nucleic acid sample in step (a) comprises mRNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid

sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and wherein the determining of step (b) comprises:

- (i) contacting the mRNA with the oligonucleotide of claim 25 under conditions permitting binding of the mRNA to the oligonucleotide so as to form a complex;
- (ii) isolating the complex so formed; and
- (iii) identifying the mRNA in the isolated complex so as to thereby determine whether the mRNA is, or is derived from, a nucleic acid which encodes the polypeptide.

59. The method of claim 57, wherein the nucleic acid sample in step (a) comprises mRNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and wherein the determining of step (b) comprises:

- (i) translating the mRNA under suitable conditions to obtain an amino acid sequence; and
- (ii) comparing the amino acid sequence of step (i) with the amino acid sequence encoded by the isolated nucleic acid of claim 9 so as to determine whether the nucleic acid sample is, or is derived from, a nucleic acid which encodes the polypeptide.

60. The method of claim 57, wherein the determining of step

(b) comprises:

- (i) amplifying the nucleic acid present in the sample of step (a); and
- (ii) detecting the presence of polypeptide in the

resulting amplified nucleic acid.

61. Use of an antibody that recognizes a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus for determining whether the subject has a predisposition for hepatocellular carcinoma, wherein such determination comprises:
- (a) obtaining an appropriate sample from the subject; and
 - (b) determining whether the sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, by contacting the sample under appropriate conditions to bind to the antibodies of claim 36 so as to determine whether the subject has a predisposition for hepatocellular carcinoma.
62. The method of claim 58, 59 or 61, wherein the oligonucleotide or antibody is labeled with a detectable marker.
63. The method of claim 62, wherein the detectable marker is a radioactive isotope, a fluorophor or an enzyme.
64. The method of claim 57, wherein the sample comprises blood, tissue or sera.
65. A method for identifying a chemical compound for the manufacture of a medicament which is capable of treating hepatocellular carcinoma which comprises:
- (a) contacting a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus,

5 such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, with the chemical compound under conditions permitting binding between the polypeptide and the chemical compound;

10 (b) detecting specific binding of the chemical compound to the polypeptide; and

(c) determining whether the chemical compound binds to the polypeptide so as to identify a chemical compound which is capable of treating hepatocellular carcinoma.

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66. A method for identifying a chemical compound for the manufacture of a medicament which is capable of preventing hepatocellular carcinoma, which comprises:

20 (a) contacting a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, with the chemical compound under conditions permitting binding between the polypeptide and the chemical compound;

25 (b) detecting specific binding of the chemical compound to the polypeptide; and

30 (c) determining whether the chemical compound binds to the polypeptide so as to identify a chemical compound which is capable of preventing hepatocellular carcinoma.

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67. A composition comprising the chemical compound identified by the method of claim 65 in an amount effective to treat hepatocellular carcinoma and a pharmaceutically effective

carrier.

- 5 68. A composition comprising the chemical compound identified by the method of claim 66 in an amount effective to prevent hepatocellular carcinoma and a pharmaceutically effective carrier.
- 10 69. Use of the composition of claim 47 or 48 as a medicament for treating hepatocellular carcinoma.
70. Use of the composition of claim 67 as a medicament for treating hepatocellular carcinoma.
- 15 71. Use of the composition of claim 47 or 48 as a medicament for preventing hepatocellular carcinoma.
72. Use of the composition of claim 67 as a medicament for preventing hepatocellular carcinoma.
- 20 73. A hepatitis vaccine, comprising a mutant form of the surface antigen of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of the major surface antigen of hepatitis B in that the amino acid at position number 145
25 of such polypeptide is an arginine rather than a glycine.
74. The vaccine of claim 73, further comprising an adjuvant.



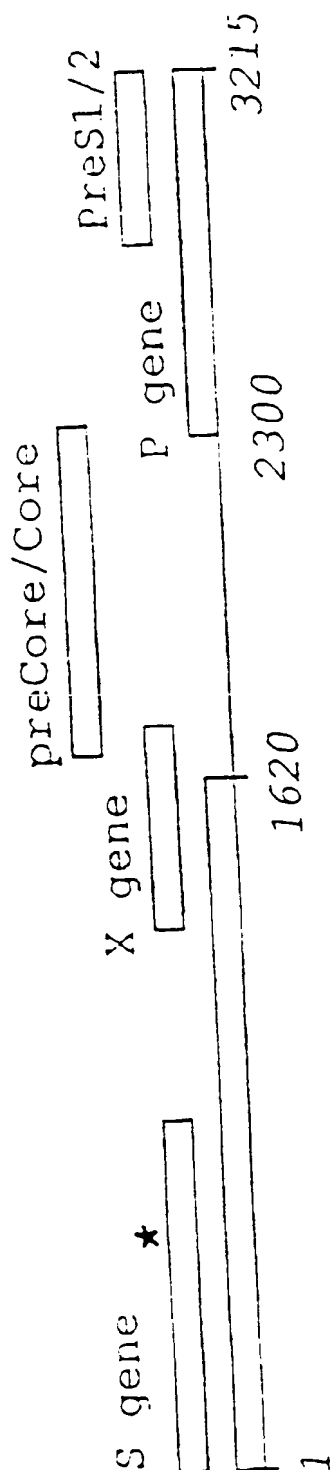


Figure 1

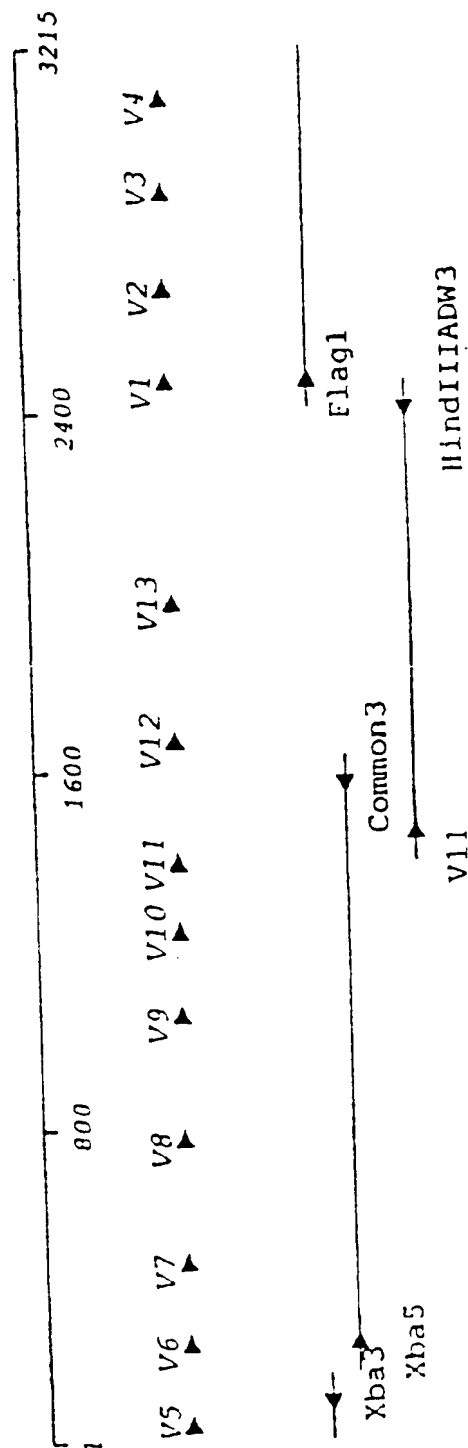


Figure 2

127 - J. PCT/PTO 13 DEC 2000

037/19533
13 DEC 2000

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) TITLE OF INVENTION: A VACCINE-INDUCED HEPATITIS B VIRAL STRAIN AND USES THEREOF

(ii) NUMBER OF SEQUENCES: 11

(2) INFORMATION FOR SEQ ID NO:1: (Figure 3)

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3215 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: circular

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

| | |
|--|------|
| CTCCACCACT TTCCACCAAA CTCTTCAAGA TCCCAGAGTC AGGGCCCTGT ACTTTCCTGC | 60 |
| TGGTGGCTCC AGTTCAGGAA CAGTGAGCCC TGCTCAGAAT ACTGTCTCTG CCATATCGTC | 120 |
| AATCTTATCG AAGACTGGGG ACCCTGTACC GAACATGGAG AACATCGCAT CAGGACTCCT | 180 |
| AGGACCCCTG CTCGTGTTAC AGGCGGGGTT TTTCTTGTTG AAAAAATCC TCACAATACC | 240 |
| GCAGAGTCTA GACTCGTGGT GGAATTCTCT CAATTTTCTA GGGGGAACAC CCGTGTGTCT | 300 |
| TGGCCAAAAT TCGCAGTCCC AAATCTCCAG TCACTCACCA ACCTGTTGTC CTCCAATTTG | 360 |
| TCCTGGTTAT CGCTGGATGT GTCTGCGGCG TTTTATCATC TTCCTCTGCA TCCTGCTGCT | 420 |
| ATGCCTCATC TTCTTGTTGG TTCTTCTGGA CTATCAAGGT ATGTTGCCCG TTTGTCTCT | 480 |
| AATTCCAGGA TCAACAACAA CCAGCACCGG ACCATGCAAA ACCTGCACAA CTCCTGCTCA | 540 |
| AGGAACCTCT ATGTTTCCCT CATGTTGCTG TACAAAACCT ACGGACAGAA ACTGCACCTG | 600 |
| TATTCCCATC CCATCATCTT GGGCTTTCCG AAAATACCTA TGGGAGTGGG CCTCAGTCCG | 660 |
| TTTCTCTTGG CTCAGTTTAC TAGTGCCATT TGTTCAAGTG TTCGTAGGGC TTTCCCCCAC | 720 |
| TGTCTGGCTT TCAGTTATAT GGATGATGTG GTTTTGGGGG CCAAGTCTGT ACAACATCTT | 780 |
| GAGTCCCTTT ATGCCGCTGT TACCAATTTT CTTTGTCTT TGGGTATACA TTAAACCCT | 840 |
| CACAAAACAA AAAGATGGGG ATATTCCCTT AACTTCATGG GATATGTCAT TGGGAGTTGG | 900 |
| GGCACATTGC CACAGGAACA TATTGTACAA AAAATCAAAA TGTGTTTGTAG GAAACTTCCT | 960 |
| GTAAACAGGC CTATTGATTG GAAAGTATGT CAACGAATTG TGGGTCTTTT GGGGTTTGCC | 1020 |
| GCCCCTTTCA CGCAATGTGG ATATCCTGCT TTAATGCCTT TATATGCATG TATACAAGCA | 1080 |
| AAACAGGCTT TTAATTTCTC GCAAACCTAC AAGACCTTTC TAAGTAAACA GTATCTGAAC | 1140 |
| CTTTACCCCG TTGCTCGGCA ACGCCCTGGT CTGTGCCAAG TGTTTGCTGA CGCAACCCCC | 1200 |
| ACTGTTGGG GCTTGGCCAT AGGCCATCAG CGCATGCGTG GAACCTTTGT GTCTCCTCTG | 1260 |



| | |
|---|------|
| CCGATCCATA CTGCGGAACT CCTAGCCGCT TGT TTTTGCTC GCAGCAGGTC TGGGGCAAAA | 1320 |
| CTCATCGGGA CTGACAATTC TGTCGTGCTC TCCCGCAAGT ATACATCATT TCCATGGCTG | 1380 |
| CTAGGCTGTG CTGCCAACTG GATCCTGCGC GGGACGTCTT TTGTTTACGT CCCGTCGGCG | 1440 |
| CTGAATCCCG CGGACGACCC CTCCCGGGGC CGCTTGGGGC TCTACGCCC GCTTCTCCGC | 1500 |
| CTGTTATACC GACCGACCAC GGGGCGCACC TCTCTTTACG CGGACTCCCC GTCTGTGCCT | 1560 |
| TCTCATCTGC CGGACCGTGT GCACTTCGCT TCACCTCTGC ACGTCGCATG GAGACCACCG | 1620 |
| TGAACGCCCCA CGGGAACCTG CCAAGGTCT TGCATAAGAG GACTCTTGGA CTTTCAGCAA | 1680 |
| TGTCAACGAC CGACCTTGAG GCATACTTCA AAGACTGTGT GTTTAATGAG TGGGAGGAGT | 1740 |
| TGGGGGAGGA GGTTAGGTTA AAGGTCTTTG TACTAGGAGG CTGTAGGCAT AAATTGGTGT | 1800 |
| GTTCAACATC ACCATGCAAC TTTTTCACCT CTGCCTAATC ATCTCATGTT CATGTCCTAC | 1860 |
| TGTTCAAGCC TCCAAGCTGT GCCTTGGGTG GCTTTGGGGC ATGGACATTG ACCCGTATAA | 1920 |
| AGAATTTGGA GCTTCTGTGG AGTTACTCTC TTTTTCGCTT TCTGACTTTT TTCCTTCTAT | 1980 |
| TCGAGATCTC CTCGACACCG CCTCTGCTCT GTATCGGGAG GCCTTAGAGT CTCCGGAACA | 2040 |
| TTGTTACCT CACCATACGG CACTCAGGCA AGCTATTCTG AGTTGGGGTG AGTTAATGAA | 2100 |
| TCTAGCCACC TGGGTGGGAA GTAATTTGGA AGATCCAGCA TCCAGGGAAT TAGTAGTCAG | 2160 |
| CTATGTCAAC GTTAATATGG GCCTAAAAAT CAGACAACTA TTGTGGTTTC ACATTTCCTG | 2220 |
| TCTTACTTTT GGGAGAGAAA CTGTTCTTGA ATATTTGGTG TCTTTTGGAG TGTGGATTCTG | 2280 |
| CACTCCTCCT GCATATAGAC CACCAAATGC CCCTATCTTA TCAACACTTC CGGAAACTAC | 2340 |
| TGTTGTTAGA CGAAGAGGCA GGTCCCCTAG AAGAAGAACT CCCTCGCCTC GCAGACGAAG | 2400 |
| GTCTCAATCG CCGCGTCGCA GAAGATCTCA ATCTCGGGAA TCTCAATGTT AGTATTCCTT | 2460 |
| GGACACATAA GGTGGGAAAC TTTACGGGGC TTTATTCTTC TACGGTACCT TGCTTTAATC | 2520 |
| CTAAATGGCA AACTCCTTCT TTTCCGGACA TTCATTTGCA GGAGGACATT CTTGATAGAT | 2580 |
| GTAAGCAATT TGTGGGGCCC CTTACAGTAA ATGAAAACAG GAGACTAAAA TTAATTATGC | 2640 |
| CTGCTAGGTT TTATCCAAAT GTTACTAAAT ATTTGCCCTT AGATAAAGGG ATCAAACCAT | 2700 |
| ATTATCCAGA GTATGTAGTT AATCATTACT TCCAGACGCG ACATTATTTA CACACTCTTT | 2760 |
| GGAAGGCGGG GATCTTATAT AAAAGAGAGT CCACACGTAG CGCCTCATTT TCGGGGTCAC | 2820 |
| CATATTCTTG GGAACAAGAT CTACAGCATG GGAGGTTGGT CTTCCAAACC TCGAAAAGGC | 2880 |
| ATGGGGACAA ATCTTTCTGT CCCCAATCCC CTGGGATTCT TCCCCGATCA TCAGTTGGAC | 2940 |
| CCTGCATTCA AAGCCAACTC AGAAAATCCA GATTGGGACC TCAACCCGCA CAAGGACAAC | 3000 |
| TGGCCGGACG CCAACAAGGT GGGAGTGGGA GCATTGGGGC CAGGGTTCAC CCCTCCTCAT | 3060 |
| GGGGGACTGT TGGGGTGGAG CCCTCAGGCT CAGGGCCTAC TCACAACTGT GCCAGCAGCT | 3120 |
| CCTCCTCCTG CCTCCACCAA TCGGCAGTCA GGAAGGCAGC CTACTCCCTT ATCTCCACCT | 3180 |
| CTAAGGGACA CTCATCCTCA GGCCATGCAG TGGAA | 3215 |



(2) INFORMATION FOR SEQ ID NO:2: (Figure 4)

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 843 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```

Met Pro Leu Ser Tyr Gln His Phe Arg Lys Leu Leu Leu Leu Asp Glu
1          5          10          15
Glu Ala Gly Pro Leu Glu Glu Glu Leu Pro Arg Leu Ala Asp Glu Gly
20          25          30
Leu Asn Arg Arg Val Ala Glu Asp Leu Asn Leu Gly Asn Leu Asn Val
35          40          45
Ser Ile Pro Trp Thr His Lys Val Gly Asn Phe Thr Gly Leu Tyr Ser
50          55          60
Ser Thr Val Pro Cys Phe Asn Pro Lys Trp Gln Thr Pro Ser Phe Pro
65          70          75          80
Asp Ile His Leu Gln Glu Asp Ile Leu Asp Arg Cys Lys Gln Phe Val
85          90          95
Glu Pro Leu Thr Val Asn Glu Asn Arg Arg Leu Lys Leu Ile Met Pro
100         105         110
Ala Arg Phe Tyr Pro Asn Val Thr Lys Tyr Leu Pro Leu Asp Lys Gly
115         120         125
Ile Lys Pro Tyr Tyr Pro Glu Tyr Val Val Asn His Tyr Phe Gln Thr
130         135         140
Arg His Tyr Leu His Thr Leu Trp Lys Ala Gly Ile Leu Tyr Lys Arg
145         150         155         160
Glu Ser Thr Arg Ser Ala Ser Phe Cys Gly Ser Pro Tyr Ser Trp Glu
165         170         175
Gln Asp Leu Gln His Gly Arg Leu Val Phe Gln Thr Ser Lys Arg His
180         185         190
Gly Asp Lys Ser Phe Cys Pro Glu Ser Pro Gly Ile Leu Pro Arg Ser
195         200         205
Ser Val Gly Pro Cys Ile Gln Ser Gln Leu Arg Lys Ser Arg Leu Gly
210         215         220
Pro Gln Pro Ala Gln Gly Gln Leu Ala Gly Arg Gln Gln Gly Gly Ser
225         230         235         240
Gly Ser Ile Arg Ala Arg Val His Pro Ser Ser Trp Gly Thr Val Gly
245         250         255
Val Glu Pro Ser Gly Ser Gly Pro Thr His Asn Cys Ala Ser Ser Ser
260         265         270
Ser Ser Cys Leu His Gln Ser Ala Val Arg Lys Ala Ala Tyr Ser Leu
275         280         285

```



Ile Ser Thr Ser Lys Gly His Ser Ser Ser Gly His Ala Val Glu Leu
 290 295 300
 His His Phe Pro Pro Asn Ser Ser Arg Ser Gln Ser Gln Gly Pro Val
 305 310 315 320
 Leu Ser Cys Trp Trp Leu Gln Phe Arg Asn Ser Glu Pro Cys Ser Glu
 325 330 335
 Tyr Cys Leu Cys His Ile Val Asn Leu Ile Glu Asp Trp Gly Pro Cys
 340 345 350
 Thr Glu His Gly Glu His Arg Ile Arg Thr Pro Arg Thr Pro Ala Arg
 355 360 365
 Val Thr Gly Gly Val Phe Leu Val Asp Lys Asn Pro His Asn Thr Ala
 370 375 380
 Glu Ser Arg Leu Val Val Asp Phe Ser Gln Phe Ser Arg Gly Asn Thr
 385 390 395 400
 Arg Val Ser Trp Pro Lys Phe Ala Val Pro Asn Leu Gln Ser Leu Thr
 405 410 415
 Asn Leu Leu Ser Ser Asn Leu Ser Trp Leu Ser Leu Asp Val Ser Ala
 420 425 430
 Ala Phe Tyr His Leu Pro Leu His Pro Ala Ala Met Pro His Leu Leu
 435 440 445
 Val Gly Ser Ser Gly Leu Ser Arg Tyr Val Ala Arg Leu Ser Ser Asn
 450 455 460
 Ser Arg Ile Asn Asn Asn Glu His Arg Thr Met Glu Asn Leu His Asn
 465 470 475 480
 Ser Cys Ser Arg Asn Leu Tyr Val Ser Leu Met Leu Leu Tyr Lys Thr
 485 490 495
 Tyr Gly Gln Lys Leu His Leu Tyr Ser His Pro Ile Ile Leu Gly Phe
 500 505 510
 Arg Lys Ile Pro Met Gly Val Gly Leu Ser Pro Phe Leu Leu Ala Gln
 515 520 525
 Phe Thr Ser Ala Ile Cys Ser Val Val Arg Arg Ala Phe Pro His Cys
 530 535 540
 Leu Ala Phe Ser Tyr Met Asp Asp Val Val Leu Gly Ala Lys Ser Val
 545 550 555 560
 Gln His Leu Glu Ser Leu Tyr Ala Ala Val Thr Asn Phe Leu Leu Ser
 565 570 575
 Leu Gly Ile His Leu Asn Pro His Lys Thr Lys Arg Trp Gly Tyr Ser
 580 585 590
 Leu Asn Phe Met Gly Tyr Val Ile Gly Ser Trp Gly Thr Leu Pro Gln
 595 600 605
 Glu His Ile Val Gln Lys Ile Lys Met Cys Phe Arg Lys Leu Pro Val
 610 615 620
 Asn Arg Pro Ile Asp Trp Lys Val Cys Gln Arg Ile Val Gly Leu Leu
 625 630 635 640



Gly Phe Ala Ala Pro Phe Thr Gln Cys Gly Tyr Pro Ala Leu Met Pro
 645 650 655
 Leu Tyr Ala Cys Ile Gln Ala Lys Gln Ala Phe Thr Phe Ser Gln Thr
 660 665 670
 Tyr Lys Thr Phe Leu Ser Lys Gln Tyr Leu Asn Leu Tyr Pro Val Ala
 675 680 685
 Arg Gln Arg Pro Gly Leu Cys Glu Val Phe Ala Asp Ala Thr Pro Thr
 690 695 700
 Gly Trp Gly Leu Ala Ile Gly His Gln Arg Met Arg Gly Thr Phe Val
 705 710 715 720
 Ser Pro Leu Pro Ile His Thr Ala Glu Leu Leu Ala Ala Cys Phe Ala
 725 730 735
 Arg Ser Arg Ser Gly Ala Lys Leu Ile Gly Thr Asp Asn Ser Val Val
 740 745 750
 Leu Ser Arg Lys Tyr Thr Ser Phe Pro Trp Leu Leu Gly Cys Ala Ala
 755 760 765
 Asn Trp Ile Leu Arg Gly Thr Ser Phe Val Tyr Val Pro Ser Ala Leu
 770 775 780
 Asn Pro Ala Asp Asp Pro Ser Arg Gly Arg Leu Gly Leu Tyr Arg Pro
 785 790 795 800
 Leu Leu Arg Leu Leu Tyr Arg Pro Thr Thr Gly Arg Thr Ser Leu Tyr
 805 810 815
 Ala Asp Ser Pro Ser Val Pro Ser His Leu Pro Asp Arg Val His Phe
 820 825 830
 Ala Ser Pro Leu His Val Ala Trp Arg Pro Pro
 835 840

(2) INFORMATION FOR SEQ ID NO:3: (Figure 5)

- (1) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 400 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met Gly Gly Trp Ser Ser Lys Pro Arg Lys Gly Met Gly Thr Asn Leu
 1 5 10 15
 Ser Val Pro Asn Pro Leu Gly Phe Phe Pro Asp His Gln Leu Asp Pro
 20 25 30
 Ala Phe Lys Ala Asn Ser Glu Asn Pro Asp Trp Asp Leu Asn Pro His
 35 40 45
 Lys Asp Asn Trp Pro Asp Ala Asn Lys Val Gly Val Gly Ala Phe Gly
 50 55 60
 Pro Gly Phe Thr Pro Pro His Gly Gly Leu Leu Gly Trp Ser Pro Gln
 65 70 75 80



Ala Gln Gly Leu Leu Thr Thr Val Pro Ala Ala Pro Pro Pro Ala Ser
 85 90 95
 Thr Asn Arg Gln Ser Gly Arg Gln Pro Thr Pro Leu Ser Pro Pro Leu
 100 105 110
 Arg Asp Thr His Pro Gln Ala Met Gln Trp Asn Ser Thr Thr Phe His
 115 120 125
 Gln Thr Leu Gln Asp Pro Arg Val Arg Ala Leu Tyr Phe Pro Ala Gly
 130 135 140
 Gly Ser Ser Ser Gly Thr Val Ser Pro Ala Gln Asn Thr Val Ser Ala
 145 150 155 160
 Ile Ser Ser Ile Leu Ser Lys Thr Gly Asp Pro Val Pro Asn Met Glu
 165 170 175
 Asn Ile Ala Ser Gly Leu Leu Gly Pro Leu Leu Val Leu Gln Ala Gly
 180 185 190
 Phe Phe Leu Leu Thr Lys Ile Leu Thr Ile Pro Gln Ser Leu Asp Ser
 195 200 205
 Trp Trp Thr Ser Leu Asn Phe Leu Gly Gly Pro Thr Val Cys Leu Gly
 210 215 220
 Gln Asn Ser Gln Ser Gln Ile Ser Ser His Ser Pro Thr Cys Cys Pro
 225 230 235 240
 Pro Ile Cys Pro Gly Tyr Arg Trp Met Cys Leu Arg Arg Phe Ile Ile
 245 250 255
 Phe Leu Cys Ile Leu Leu Leu Cys Leu Ile Phe Leu Leu Val Leu Leu
 260 265 270
 Asp Tyr Gln Gly Met Leu Pro Val Cys Pro Leu Ile Pro Gly Ser Thr
 275 280 285
 Thr Thr Ser Thr Gly Pro Cys Lys Thr Cys Thr Thr Pro Ala Gln Gly
 290 295 300
 Thr Ser Met Phe Pro Ser Cys Cys Cys Thr Lys Pro Thr Asp Arg Asn
 305 310 315 320
 Cys Thr Cys Ile Pro Ile Pro Ser Ser Trp Ala Phe Ala Lys Tyr Leu
 325 330 335
 Trp Glu Trp Ala Ser Val Arg Phe Ser Trp Leu Ser Leu Leu Val Pro
 340 345 350-
 Phe Val Gln Trp Phe Val Gly Leu Ser Pro Thr Val Trp Leu Ser Val
 355 360 365
 Ile Trp Met Met Trp Phe Trp Gly Pro Ser Leu Tyr Asn Ile Leu Ser
 370 375 380
 Pro Phe Met Pro Leu Leu Pro Ile Phe Phe Cys Leu Trp Val Tyr Ile
 385 390 395 400



(2) INFORMATION FOR SEQ ID NO:4: (Figure 6)

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 212 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```

Met Gln Leu Phe His Leu Cys Leu Ile Ile Ser Cys Ser Cys Pro Thr
 1              5              10              15
Val Gln Ala Ser Lys Leu Cys Leu Gly Trp Leu Trp Gly Met Asp Ile
 20              25              30
Asp Pro Tyr Lys Glu Phe Gly Ala Ser Val Glu Leu Leu Ser Phe Leu
 35              40              45
Pro Ser Asp Phe Phe Pro Ser Ile Arg Asp Leu Leu Asp Thr Ala Ser
 50              55              60
Ala Leu Tyr Arg Glu Ala Leu Glu Ser Pro Glu His Cys Ser Pro His
 65              70              75              80
His Thr Ala Leu Arg Gln Ala Ile Leu Ser Trp Gly Glu Leu Met Asn
 85              90              95
Leu Ala Thr Trp Val Gly Ser Asn Leu Glu Asp Pro Ala Ser Arg Glu
100              105              110
Leu Val Val Ser Tyr Val Asn Val Asn Met Gly Leu Lys Ile Arg Gln
115              120              125
Leu Leu Trp Phe His Ile Ser Cys Leu Thr Phe Gly Arg Glu Thr Val
130              135              140
Leu Glu Tyr Leu Val Ser Phe Gly Val Trp Ile Arg Thr Pro Pro Ala
145              150              155              160
Tyr Arg Pro Pro Asn Ala Pro Ile Leu Ser Thr Leu Pro Glu Thr Thr
165              170              175
Val Val Arg Arg Arg Gly Arg Ser Pro Arg Arg Arg Thr Pro Ser Pro
180              185              190
Arg Arg Arg Arg Ser Gln Ser Pro Arg Arg Arg Arg Ser Gln Ser Arg
195              200              205
Glu Ser Gln Cys
210

```

(2) INFORMATION FOR SEQ ID NO:5: (Figure 7)

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 154 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear



(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Ala Ala Arg Leu Cys Cys Gln Leu Asp Pro Ala Arg Asp Val Leu
 1 5 10 15
 Cys Leu Arg Pro Val Gly Ala Glu Ser Arg Gly Arg Pro Leu Pro Gly
 20 25 30
 Pro Leu Gly Ala Leu Pro Pro Ala Ser Pro Pro Val Ile Pro Thr Asp
 35 40 45
 His Gly Ala His Leu Ser Leu Arg Gly Leu Pro Val Cys Ala Phe Ser
 50 55 60
 Ser Ala Gly Pro Cys Ala Leu Arg Phe Thr Ser Ala Arg Arg Met Glu
 65 70 75 80
 Thr Thr Val Asn Ala His Gly Asn Leu Pro Lys Val Leu His Lys Arg
 85 90 95
 Thr Leu Gly Leu Ser Ala Met Ser Thr Thr Asp Leu Glu Ala Tyr Phe
 100 105 110
 Lys Asp Cys Val Phe Asn Glu Trp Glu Glu Leu Gly Glu Glu Val Arg
 115 120 125
 Leu Lys Val Phe Val Leu Gly Gly Cys Arg His Lys Leu Val Cys Ser
 130 135 140
 Pro Ser Pro Cys Asn Phe Phe Thr Ser Ala
 145 150

(2) INFORMATION FOR SEQ ID NO:6: (Figure 8)

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 35 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ATAAGCTTATG CCCCTATCTT ATCAACACTT CCGGA

35

(2) INFORMATION FOR SEQ ID NO:7: (Figure 9)

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 25 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GAGTCTAGAC TCTGCGGTAT TGTGA

25



(2) INFORMATION FOR SEQ ID NO:8: (Figure 10)

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GAGTCTAGAC TCGTGGTGGA CTTCT

25

(2) INFORMATION FOR SEQ ID NO:9: (Figure 11)

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TGAGAATTCT CACGGTGGTC TCCATGCGAC GT

32

(2) INFORMATION FOR SEQ ID NO:10: (Figure 12)

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TTTGTTTACG TCCCGT

16

(2) INFORMATION FOR SEQ ID NO:11: (Figure 13)

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CTAAGCTTAG TTTCCGGAAG TGTTGAT

27



INTERNATIONAL SEARCH REPORT

International application No.
PCT/98/00045

| | | |
|---|--|---|
| A. CLASSIFICATION OF SUBJECT MATTER IPC ⁶ : C 12 N 15/51,7/01; C 07 K 14/02,16/08; C 12 Q 1/68; A 61 K 39/29 According to International Patent Classification (IPC) or to both national classification and IPC | | |
| B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC ⁶ : C 12 N 15/51,7/01; C 07 K 14/02,16/08; C 12 Q 1/68; A 61 K 39/29 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched | | |
| Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) WPI, EPODOC | | |
| C. DOCUMENTS CONSIDERED TO BE RELEVANT | | |
| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| A | WO 95/21 189 A1 (IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY & MEDICINE) 10 August 1995 (10.08.95), claims 1,10-16. | 1,12,19,35,73 |
| A | WO 98/11 916 A1 (THE BOARD OF REGENTS OF THE UNIVERSITY OF TEXAS SYSTEM) 26 March 1998 (26.03.98), claims 1,5,6,7,15,19. | 1,12,19 |
| <div style="display: flex; justify-content: space-between;"> <input type="checkbox"/> Further documents are listed in the continuation of Box C. <input checked="" type="checkbox"/> See patent family annex. </div> | | |
| <div style="display: flex;"> <div style="flex: 1;"> <p>* Special categories of cited documents:</p> <p>„A“ document defining the general state of the art which is not considered to be of particular relevance</p> <p>„E“ earlier application or patent but published on or after the international filing date</p> <p>„L“ document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>„O“ document referring to an oral disclosure, use, exhibition or other means</p> <p>„P“ document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="flex: 1;"> <p>„T“ later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>„X“ document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>„Y“ document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>„&“ document member of the same patent family</p> </div> </div> | | |
| Date of the actual completion of the international search <div style="text-align: center;">08 March 1999 (08.03.99)</div> | | Date of mailing of the international search report <div style="text-align: center;">17 March 1999 (17.03.99)</div> |
| Name and mailing address of the ISA/AT Austrian Patent Office Kohlmarkt 8-10; A-1014 Vienna Facsimile No. 1/53424/535 | | Authorized officer <div style="text-align: center;">Wolf</div> Telephone No. 1/53424/436 |

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SG 98/00045

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 51-54 and 69-72

~~because they relate to subject matter not required to be searched by this Authority, namely:~~

Although these claims concern a method of treatment of the human or animal body by therapy (Rule 31.1 (iv) PCT) the search has been carried out and based on the alleged effects.

2. ☐ Claims Nos.:

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐

The additional search fees were accompanied by the applicant's protest.

☐

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/SG 98/00045

| In Recherchenbericht angeführtes Patentedokument Patent document cited in search report Document de brevet cité dans le rapport de recherche | Datum der Veröffentlichung Publication date Date de publication | Mitglied(er) der Patentfamilie Patent family member(s) Membre(s) de la famille de brevets | Datum der Veröffentlichung Publication date Date de publication |
|---|--|---|--|
| WD A1 9521189 | 10-08-95 | AU A1 15426/95 AU B2 693154 CA AA 2182375 CN A 1143373 EP A1 741745 GB AD 9401987 JP T2 9508284 NZ A 278937 PL A1 315784 SG A1 49631 US A 5856084 | 21-08-95 25-06-98 10-08-95 19-02-97 13-11-96 30-03-94 26-08-97 26-06-98 09-12-96 15-06-98 05-01-99 |
| WD A1 9811916 | 26-03-98 | AU A1 43538/97 | 14-04-98 |

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

| | | |
|--|---|---|
| Applicant's or agent's file reference PCT/8013374 | FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below. | |
| International application No. PCT/SG 98/00045 | International filing date (day/month/year) 19 June 1998 (19.06.98) | (Earliest) Priority Date (day/month/year) |
| Applicant GOVERNMENT OF THE REPUBLIC OF SINGAPORE et al. | | |

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 4 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

- a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

- b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing:

☒ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

2. ☐ **Certain claims were found unsearchable** (See Box I).

3. ☐ **Unity of invention is lacking** (See Box II).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.: _____

☐ as suggested by the applicant.

☒ None of the figures.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.



INTERNATIONAL SEARCH REPORT

International application No.

T/SG 98/00045

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 51-54 and 69-72
~~because they relate to subject matter not required to be searched by this Authority, namely:~~
Although these claims concern a method of treatment of the human or animal body by therapy (Rule 31.1 (iv) PCT) the search has been carried out and based on the alleged effects.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.



INTERNATIONAL SEARCH REPORT

International application No.

PC 88G 98/00045

A. CLASSIFICATION OF SUBJECT MATTER

IPC⁶: C 12 N 15/51,7/01; C 07 K 14/02,16/08; C 12 Q 1/68; A 61 K 39/29

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC⁶: C 12 N 15/51,7/01; C 07 K 14/02,16/08; C 12 Q 1/68; A 61 K 39/29

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, EPODOC

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|-----------|--|-----------------------|
| ---A--- | WO 95/21 189 A1 (IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY & MEDICINE) 10 August 1995 (10.08.95), claims 1,10-16. | 1,12,19,35,73 |
| A | WO 98/11 916 A1 (THE BOARD OF REGENTS OF THE UNIVERSITY OF TEXAS SYSTEM) 26 March 1998 (26.03.98), claims 1,5,6,7,15,19. | 1,12,19 |
| | ---- | |

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:

- „A“ document defining the general state of the art which is not considered to be of particular relevance
- „E“ earlier application or patent but published on or after the international filing date
- „L“ document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- „O“ document referring to an oral disclosure, use, exhibition or other means
- „P“ document published prior to the international filing date but later than the priority date claimed

- „T“ later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- „X“ document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- „Y“ document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- „&“ document member of the same patent family

Date of the actual completion of the international search

08 March 1999 (08.03.99)

Date of mailing of the international search report

17 March 1999 (17.03.99)

Name and mailing address of the ISA/AT
Austrian Patent Office
Kohlmarkt 8-10; A-1014 Vienna
Facsimile No. 1/53424/535

Authorized officer

Wolf

Telephone No. 1/53424/436



INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/SG 98/00045

| In Recherchenbericht angeführtes Patentdokument Patent document cited in search report Document de brevet cité dans le rapport de recherche | Datum der Veröffentlichung Publication date Date de publication | Mitglied(er) der Patentfamilie Patent family member(s) Membre(s) de la famille de brevets | Datum der Veröffentlichung Publication date Date de publication |
|--|--|---|--|
| WD A1 9521189 | 10-08-95 | AU A1 15426/95 AU B2 693154 CA AA 2182375 CN A 1143373 EP A1 741745 GB A0 9401987 JP T2 9508284 NZ A 278937 PL A1 315784 SG A1 49631 US A 5856084 | 21-08-95 25-06-98 10-08-95 19-02-97 13-11-96 30-03-94 26-08-97 26-06-98 09-12-96 15-06-98 05-01-99 |
| WD A1 9811916 | 26-03-98 | AU A1 43538/97 | 14-04-98 |

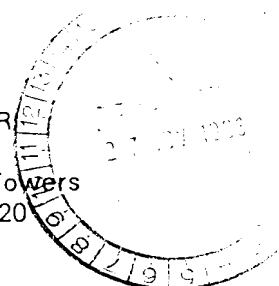


PCT

NOTIFICATION OF THE RECORDING
OF A CHANGE(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

DREW & NAPIER
20 Raffles Place
#17-00 Ocean Towers
Singapore 048620
SINGAPOUR

| | |
|---|---|
| Date of mailing (day/month/year) 12 November 1998 (12.11.98) | IMPORTANT NOTIFICATION |
| Applicant's or agent's file reference PCT/8013374 | |
| International application No. PCT/SG98/00045 | International filing date (day/month/year) 19 June 1998 (19.06.98) |

1. The following indications appeared on record concerning:

☒ the applicant ☒ the inventor ☐ the agent ☐ the common representative

Name and Address

LEONG, Ai, Lin
Block 119, Toa Payoh
Lorong 1, #04-443
Singapore 310119
Singapore

State of Nationality

State of Residence

Telephone No.

Facsimile No.

Teleprinter No.

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☐ the person ☐ the name ☒ the address ☐ the nationality ☐ the residence

Name and Address

LEONG, Ai, Lin
Block 263 Yishun Street 22
12-161
Singapore 760263
Singapore

State of Nationality

State of Residence

Telephone No.

Facsimile No.

Teleprinter No.

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

| | |
|--|---|
| <input checked="" type="checkbox"/> the receiving Office | <input type="checkbox"/> the designated Offices concerned |
| <input checked="" type="checkbox"/> the International Searching Authority | <input type="checkbox"/> the elected Offices concerned |
| <input type="checkbox"/> the International Preliminary Examining Authority | <input type="checkbox"/> other: |

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

S. Baharlou

Telephone No.: (41-22) 338.83.38



PATENT COOPERATION TREATY

09/01/1998

From the:
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

GIRVIN, Cecelia et al.
DREW and NAPIER
20 Raffles Place
17-00 Ocean Towers
SINGAPORE 048620
SINGAPOUR

PCT

WRITTEN OPINION

(PCT Rule 66)

Date of mailing
(day/month/year)

10.03.00

Applicant's or agent's file reference

CG/PHM/PAT/8013374

REPLY DUE

within 3 month(s)
from the above date of mailing

International application No.

PCT/SG98/00045

International filing date (day/month/year)

19/06/1998

Priority date (day/month/year)

19/06/1998

International Patent Classification (IPC) or both national classification and IPC

C12N15/51

Applicant

GOVERNMENT OF REPUBLIC OF SINGAPORE ... et al.

1. This written opinion is the **first** drawn up by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

- I ☒ Basis of the opinion
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☒ Lack of unity of invention
- V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain document cited
- VII ☒ Certain defects in the international application
- VIII ☒ Certain observations on the international application

3. The applicant is hereby **invited to reply** to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also: For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 bis.
For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 19/10/2000.

Name and mailing address of the international preliminary examining authority.



European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx 523656 epmu d
Fax +49 89 2399 - 4465

Authorized officer / Examiner

SCHEFFZYK, I

Formalities officer (incl. extension of time limits)

Vullo, C

Telephone No. +49 89 2399 8061





I. Basis of the opinion

1. This opinion has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed"*):

Description, pages:

1-35 as originally filed

Claims, No.:

1-74 as originally filed

Drawings, sheets:

1/2,2/2 as originally filed

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. This opinion has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

Additional observations, if necessary:

IV. Lack of unity of invention

1. In response to the invitation (Form PCT/IPEA/405) to restrict or pay additional fees, the applicant has:

- ☐ restricted the claims.
☐ paid additional fees.
☐ paid additional fees under protest.
☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied with for the following reasons



and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees:

see separate sheet

3. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this opinion:

- ☒ all parts.
- ☐ the parts relating to claims Nos. .

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

| | | |
|-------------------------------|--------|--|
| Novelty (N) | Claims | 1, 21-24, 31-34, 36, 47, 48-50, 73, 74 |
| Inventive step (IS) | Claims | 1-74 |
| Industrial applicability (IA) | Claims | 51-54: see section VIII/8). |

2. Citations and explanations

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet



SECTION IV-----

The IPEA is of the opinion that present application lacks unity a priori since the subject-matter of present claims is not directed to one single invention as required by Rule 13.1-13.3 PCT but covers two separate inventions not linked by a common inventive concept, namely:

- invention 1: major surface antigen of hepatitis B virus having at amino acid residue 145 arginine instead of glycine (mutated form of the major surface antigen of hepatitis B virus) (claims 1-9, 10, 12-22, 24-30, 35, 37-47, 49-74 complete) and
- invention 2: major surface antigen of hepatitis B virus which does not necessarily have at amino acid residue 145 arginine in place of glycine but may have glycine instead of arginine at said position (wild-type form of the major surface antigen) (claims 11, 23, 31-34, 36, 48 complete)

SECTION V-----

Novelty:

It is evident from the date of deposit defined in claim 1 (15.12.97) that the strain according to claim 1 already was available before the priority date of present application. Correspondingly, claim 1 lacks novelty (Art. 33(2) PCT). Moreover, from the description of present application (see page 2, second paragraph) it appears that the major surface antigen of hepatitis B virus having at amino acid residue 145 arginine in place of glycine also was already available before the filing (priority) date of present application. Therefore, novelty of claims 21, 22, 24, 47, 73 and 74 cannot be acknowledged either. With respect to claims 73 and 74 it is pointed out that an indication of use in a product claim is not considered suitable to limit the scope of such a claim (cf. Guidelines C-III 4.8 PCT).

Claims 11, 23, 31-33, 34, 36 and 48 cannot be considered to be novel since



the sequence defined in these claims corresponds to the amino acid sequence of the wild-type major surface antigen of hepatitis B virus. Consequently, these claims embrace nucleic acid molecules encoding the wild-type major surface antigen of hepatitis B virus, the wild-type major surface antigen per se, antibodies directed against said wild-type protein and methods for preparing such antibodies.

In addition, claims 49, 50 also lack novelty since at present it cannot be ruled out that any readily available compound may be identified by the methods defined in claims 45, 46, 65 and 66.

Inventive step:

The existence of the hepatitis B virus strain with the mutation at position 145 (arginine instead of glycine) was already known at the priority date of present application (see above). Moreover, the association of said strain with the development of acute hepatitis B also is known in the art (see page 2, last paragraph of present application). Thus, the provision of the mutated major surface antigen of said strain and/or nucleic acid encoding it and the use thereof in detection systems and/or screening methods appear to have been obvious to a person skilled in the art. Therefore, present claims lack inventive activity and thus do not meet the requirements of Art. 33(3) PCT.

SECTION VII-----

- 1). With respect to the expression "incorporated by reference" written in the specification Applicant's attention is drawn to Guidelines C-II 4.3 and C-II 4.18 PCT.

SECTION VIII-----

- 1). With respect to claims 27-32 it is noted that it is questionable whether the methods defined in these claims actually contain all features essential to obtain antibodies exclusively binding to the mutated major surface antigen of hepatitis B



virus which only differs from the corresponding wild-type protein in that it has arginine at position 145 instead of glycine. Relating to this it is noted that step e) defined in claims 27 and 31 also covers the detection of antibodies binding to regions of the wild-type major surface antigen! Moreover, the residues defined in claims 29 and 31 correspond to those of the wild-type major surface antigen.

- 2). The scope of claims 45, 46, 65, 66 is unclear since it is not known whether said claims are directed to a method of identifying a chemical compound or whether they are directed to the use of chemical compounds in a method for the manufacture of a medicament.
- 3). The term "derivative thereof" used in claims 47 and 48 renders the scope of said claims unclear (Art. 6 PCT) since it is not clear which peptides are covered by said expression and which are not.
- 4). Claims 49, 50, 67 and 68 are not supported by the specification since the specification fails to mention any chemical compound identified by the method according to claims 45, 46, 65 and 66 (Art. 6 PCT in combination with Guidelines C-III 6.3 PCT). Moreover, with respect to these claims an objection under Art. 5 PCT also arises since in the absence of evidence showing that chemical compounds suitable to exclusively bind to the mutant major surface antigen having at position 145 arginine in place of glycine actually exist the existence of such compounds is merely speculative and thus it is questionable whether a person skilled in the art actually is able to prepare the claimed compositions.
- 5). The same objections apply equally to claims 52 and 46 relating to the use of the compositions according to claims 49, 50, 67 and 68.
- 6). The reference in claims 52, 54 and 62 should be checked (claims 45 and 46 are not directed to compositions but to methods and claim 61 does not relate to a method but to a use!)
- 7). Claims 51-54, 57-64, 69-72 are not supported by the specification since the application as originally filed fails to provide any facts and data demonstrating that the mutant form (Arg instead of Gly at position 145) of the major surface antigen is



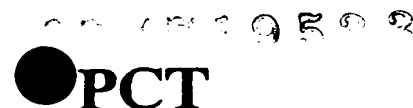
actually suitable for the medical applications defined in these claims. Relating to this it is also pointed out that there is no evidence in the application showing a relationship of the occurrence of said mutant form with the development of HCC (hepatocellular carcinoma).

- 8). Claims 51-54 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).



PATENT COOPERATION TREATY

From the RECEIVING OFFICE



To:

Drew & Napier
Robinson Road
P O Box 152
Singapore 900302

INVITATION TO CORRECT DEFECTS IN THE INTERNATIONAL APPLICATION

(PCT Articles 3(4)(i) and 14(1) and Rule 26)

| | |
|--|--|
| <p>Date of mailing (day/month/year) 27 JUN 1998</p> | |
| <p>Applicant's or agent's file reference PCT/SG98/00045</p> | <p>REPLY DUE within 1 months/days from the above date of mailing</p> |
| <p>International application No. PCT/SG98/00045</p> | <p>International filing date (day/month/year) 19 JUNE 1998 (19.06.98)</p> |
| <p>Applicant GOVERNMENT OF THE REPUBLIC OF SINGAPORE</p> | |

The applicant is hereby invited, within the time limit indicated above, to correct the defects in the international application, which are specified on the attached

☒ Annex A

☒ Annex B

☒ Annex C

Additional observations (if necessary):

HOW TO CORRECT THE DEFECTS?

Correction must be submitted by filing a replacement sheet embodying the correction and a letter accompanying the replacement sheet, which shall draw attention to the difference between the replaced sheet and the replacement sheet. A correction may be stated in a letter only if it is of such a nature that it can be transferred from the letter to the record copy without adversely affecting the clarity and direct reproducibility of the sheet onto which the correction is to be transferred (Rule 26.4(a)).

ATTENTION

Failure to correct the defects will result in the international application being considered withdrawn by this receiving Office (see Rule 26.5 for further details).

A copy of this invitation and any attachments has been sent to the International Bureau

☒ and the International Searching Authority.

| | |
|---|---|
| <p>Name and mailing address of the receiving Office</p> <p style="text-align: center;">Registry of Patents 31 Bras Basah Road #04-01 Raffles City The Park Singapore 0718</p> <p>Facsimile No. 339-0252</p> | <p>Authorized officer MDM MISLIA BTE BUKHON</p> <p style="text-align: center;">Registry of Patents</p> <p>Telephone No. Tel: 330-2743</p> |
|---|---|



The receiving Office has found the following defects in the international application:

1. As to signature* of the international application (Rules 4.15 and 90.4), the request:

- a. ☐ is not signed.
- b. ☐ is not signed by all the applicants.
- c. ☐ is not accompanied by the statement referred to in the check list in Box No. VIII of the request explaining the lack of the signature of an applicant for the designation of the United States of America.
- d. ☒ is signed by what appears to be an agent/common representative but
 - ☐ the international application is not accompanied by a power of attorney appointing him.
 - ☒ the power of attorney accompanying the international application was not signed by all the applicants.
applicants/inventors for US
- e. ☐ other (specify):

* All applicants must sign, including inventors if they are also applicants (e.g. where the United States of America is designated).

2. As to indications concerning the applicant, the request (Rules 4.4 and 4.5):

- a. ☐ does not properly indicate the applicant's name (specify):
- b. ☐ does not indicate the applicant's address.
- c. ☐ does not properly indicate the applicant's address (specify):
- d. ☐ does not indicate the applicant's nationality.
- e. ☐ does not indicate the applicant's residence.
- f. ☐ other (specify):

3. As to the language of some parts of the international application (Rule 12.1):

- a. ☐ the request is not in (one of) the admitted language(s) which is (are): _____
- b. ☐ the text matter of the drawings is not in (one of) the admitted language(s) which is (are): _____
- c. ☐ the abstract is not in (one of) the admitted language(s) which is (are): _____

4. The title of the invention:

- a. ☐ is not indicated in Box No. I of the request (Rule 4.1(a)).
- b. ☐ is not indicated at the top of the first sheet of the description (Rule 5.1(a)).
- c. ☐ as appearing in Box No. I of the request is not identical with the title heading the description (Rule 5.1(a)).

5. As to the abstract (Rule 8):

- ☐ the international application does not contain an abstract.



The physical requirements of the international application are not complied with to the extent which is necessary for the purpose of a reasonably uniform international publication, as specified below (Rule 11). The receiving Office has found the following defects in the presentation of the text matter of the international application:

| | Request | Description | Claims | Abstract |
|--|--------------------------|--------------------------|--------------------------|--------------------------|
| a. <input type="checkbox"/> The sheets do not admit of direct reproduction. | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| b. <input type="checkbox"/> The element does not commence on a new sheet. | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| c. <input type="checkbox"/> Sheets are not free from creases, cracks, folds. | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| d. <input type="checkbox"/> Sheets are not used in the upright position. | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| e. <input type="checkbox"/> One side of the sheets is not left unused. | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| f. <input type="checkbox"/> The paper of the sheets is not flexible/strong/white/smooth/non-shiny/durable. | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| g. <input type="checkbox"/> The sheets are not connected as prescribed (Rule 11.4(b)). | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| h. <input type="checkbox"/> Sheets are not A4 size (29.7cm x 21cm). | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| i. <input type="checkbox"/> The minimum margins on the sheets are not as prescribed (top: 2cm; left side: 2.5cm; right side: 2cm; bottom: 2cm). | | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| j. <input type="checkbox"/> The file reference number indicated on the sheets does not appear in the left-hand corner of the sheets, within 1.5 cm of the top of the sheets. | | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| k. <input type="checkbox"/> The file reference number exceeds the maximum of 12 characters. | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| l. <input type="checkbox"/> The sheets of the description, claims and abstract are not numbered in consecutive Arabic numerals. | | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| m. <input type="checkbox"/> The sheet numbers are not centered at the top or bottom of the sheets. | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| n. <input type="checkbox"/> The sheet numbers are in the margin (see i. above for the size of the margins). | | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| o. <input type="checkbox"/> The text matter is not typed or printed. | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| p. <input type="checkbox"/> The typing on the sheets is not 1½-spaced. | | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| q. <input type="checkbox"/> The characters in the text matter on the sheets are less than 0.21 cm high in capital letters. | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| r. <input type="checkbox"/> The text matter on the sheets is not in dark, indelible color. | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| s. <input type="checkbox"/> The element contains drawings. | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| t. <input type="checkbox"/> The sheets contain alterations/overwritings/interlineations/too many erasures. | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| u. <input type="checkbox"/> The sheets contain photocopy marks. | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |

Further observations (if necessary):

* The international application should be placed in the following order: the request, the description, the claims, the abstract and the drawings.



The physical requirements of the international application are not complied with to the extent which is necessary for the purpose of a reasonably uniform international publication, as specified below (Rule 11). The receiving Office has found the following defects in the presentation of the drawings of the international application:

I. In regard to the sheets containing drawings:

- a. ☐ the sheets do not admit of direct reproduction.
- b. ☐ the sheets are not free from creases, cracks, folds.
- c. ☐ one side of the sheets is not left unused.
- d. ☐ the paper of the sheets is not flexible/strong/white/smooth/non-shiny/durable.
- e. ☐ the drawings do not commence on a new sheet.
- f. ☐ the sheets are not connected as prescribed (Rule 11.4(b)).
- g. ☐ the sheets are not A4 size (29.7cm x 21cm).
- h. ☐ the minimum margins on the sheets are not as prescribed (top: 2.5cm; left side: 2.5cm; right side: 1.5cm; bottom: 1cm).
- i. ☐ the file reference number indicated on the sheets does not appear in the left-hand corner of the sheets, within 1.5 cm of the top of the sheets.
- j. ☐ the file reference number exceeds the maximum of 12 characters.
- k. ☐ the sheets are not free from frames around usable or used surfaces.
- l. ☒ the sheets are not numbered in consecutive Arabic numerals (e.g. 1/3, 2/3, 3/3).
- m. ☐ the sheet numbers are not centered at the top or bottom of the sheets.
- n. ☐ the sheet numbers are in the margin (see h. above for the size of the margins).
- o. ☐ the sheets contain alterations/overwritings/interlineations/too many erasures.
- p. ☐ the sheets contain photocopy marks.

II. The drawings (Rule 11.13):

- a. ☐ do not admit of direct reproduction.
- b. ☐ contain unnecessary text matter.
- c. ☐ contain words so placed as to prevent translation without interference with lines thereof.
- d. ☐ are not executed in durable black color; the lines are not uniformly thick and well-defined.
- e. ☐ contain cross-sections not properly hatched.
- f. ☐ would not be properly distinguishable in reduced reproduction.
- g. ☐ contain scales not represented graphically.
- h. ☐ contain numbers, letters and reference lines lacking simplicity and clarity.
- i. ☐ contain lines drafted without the aid of drafting instruments.
- j. ☐ contain disproportionate elements of a figure not necessary for clarity.
- k. ☐ contain numbers and letters of height less than 0.32 cm.
- l. ☐ contain letters not conforming to the Latin, and where customary, Greek alphabets.
- m. ☐ contain figures on two or more sheets which form a single complete figure but which are not able to be assembled without concealing parts thereof.
- n. ☐ contain figures which are not properly arranged and clearly separated.
- o. ☐ contain different figures not numbered in consecutive Arabic numerals.
- p. ☐ contain different figures not numbered independent of the numbering of the sheets.
- q. ☐ are not restricted to reference signs mentioned in the description.
- r. ☐ do not contain reference signs that are mentioned in the description.
- s. ☐ contain the same feature denoted by different reference signs.

Further observations (if necessary):



PATENT COOPERATION TREATY

From the RECEIVING OFFICE

06719533
PCT

To:

Drew & Napier
Robinson Road
P O Box 152
Singapore 900302

NOTIFICATION OF THE INTERNATIONAL APPLICATION NUMBER AND OF THE INTERNATIONAL FILING DATE

(PCT Rule 20.5(c))

Date of mailing (day/month/year) **27 JUN 1998**

Applicant's or agent's file reference
PCT/SG98/00049

IMPORTANT NOTIFICATION

International application No.
PCT/SG98/00049

International filing date (day/month/year)
19 JUNE 1998 (19.06.98)

Priority date (day/month/year)
-

Applicant **GOVERNMENT OF REPUBLIC OF SINGAPORE**

Title of the invention **A vaccine-induced hepatitis B viral strain and uses thereof**

1. The applicant is hereby notified that the international application has been accorded the international application number and the international filing date indicated above.

2. The applicant is further notified that the record copy of the international application:



was transmitted to the International Bureau on

27 JUN 1998



has not yet been transmitted to the International Bureau for the reason indicated below and a copy of this notification has been sent to the International Bureau*:



because the necessary national security clearance has not yet been obtained.



because (reason to be specified):

* The International Bureau monitors the transmittal of the record copy by the receiving Office and will notify the applicant (with Form PCT/IB/301) of its receipt. Should the record copy not have been received by the expiration of 14 months from the priority date, the International Bureau will notify the applicant (Rule 22.1(c)).

Name and mailing address of the receiving Office

Registry of Patents
51 Bras Basah Road #04-01
Plaza By The Park

Facsimile No.

Authorized officer

MDM MISLIA BTE BUKHON
Registry of Patents
Tel: 330-2743

Telephone No.

Form PCT/RO/105 (July 1992) Singapore 0718
Fax No: 339-0252
Tel: 330-2743



CORRECTED
VERSION
PCT

09/719533
LTA
TENT COOPERATION TREATY

PCT/SG98/00045

From the INTERNATIONAL BUREAU

To:

DREW & NAPIER
20 Raffles Place
#17-00 Ocean Towers
Singapore 048620
SINGAPOUR

Date of mailing (day/month/year)
17 August 1998 (17.08.98)

IMPORTANT NOTIFICATION

Applicant's or agent's file reference
PCT/8013374

International application No.
PCT/SG98/00045

The applicant is hereby notified that the International Bureau has received the record copy of the international application as detailed below.

Name(s) of the applicant(s) and State(s) for which they are applicants:

GOVERNMENT OF THE REPUBLIC OF SINGAPORE (for all designated States except US)
QON, Chong, Jin et al (for US)

International filing date : 19 June 1998 (19.06.98)

Priority date(s) claimed :

Date of receipt of the record copy
by the International Bureau : 01 July 1998 (01.07.98)

List of designated Offices :

AP : GH,GM,KE,LS,MW,SD,SZ,UG,ZW

EA : AM,AZ,BY,KG,KZ,MD,RU,TJ,TM

EP : AT,BE,CH,CY,DE,DK,ES,FI,FR,GB,GR,IE,IT,LU,MC,NL,PT,SE

OA : BF,BJ,CF,CG,CI,CM,GA,GN,ML,MR,NE,SN,TD,TG

National : AL,AM,AT,AU,AZ,BA,BB,BG,BR,BY,CA,CH,CN,CU,CZ,DE,DK,EE,ES,FI,GB,GE,GH,GM,
GW,HU,ID,IL,IS,JP,KE,KG,KP,KR,KZ,LC,LK,LR,LS,LT,LU,LV,MD,MG,MK,MN,MW,MX,NO,NZ,PL,
PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TR,TT,UA,UG,US,UZ,VN,YU,ZW

ATTENTION

The applicant should carefully check the data appearing in this Notification. In case of any discrepancy between these data and the indications in the international application, the applicant should immediately inform the International Bureau.

In addition, the applicant's attention is drawn to the information contained in the Annex, relating to:

- ☒ time limits for entry into the national phase
☐ confirmation of precautionary designations
☐ requirements regarding priority documents

A copy of this Notification is being sent to the receiving Office and to the International Searching Authority.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Authorized officer:

S. Baharlou

Facsimile No. (41-22) 740.14.35

Telephone No. (41-22) 338.83.38



INFORMATION ON TIME LIMITS FOR ENTERING THE NATIONAL PHASE

The applicant is reminded that the "national phase" must be entered before each of the designated Offices indicated in the Notification of Receipt of Record Copy (Form PCT/IB/301) by paying national fees and furnishing translations, as prescribed by the applicable national laws.

The time limit for performing these procedural acts is **20 MONTHS** from the priority date or, for those designated States which the applicant elects in a demand for international preliminary examination or in a later election, **30 MONTHS** from the priority date, provided that the election is made before the expiration of 19 months from the priority date. Some designated (or elected) Offices have fixed time limits which expire even later than 20 or 30 months from the priority date. In other Offices an extension of time or grace period, in some cases upon payment of an additional fee, is available.

In addition to these procedural acts, the applicant may also have to comply with other special requirements applicable in certain Offices. It is the applicant's responsibility to ensure that the necessary steps to enter the national phase are taken in a timely fashion. Most designated Offices do not issue reminders to applicants in connection with the entry into the national phase.

For detailed information about the procedural acts to be performed to enter the national phase before each designated Office, the applicable time limits and possible extensions of time or grace periods, and any other requirements, see the relevant Chapters of Volume II of the PCT Applicant's Guide. Information about the requirements for filing a demand for international preliminary examination is set out in Chapter IX of Volume I of the PCT Applicant's Guide.

GR and ES became bound by PCT Chapter II on 7 September 1996 and 6 September 1997, respectively, and may, therefore, be elected in a demand or a later election filed on or after 7 September 1996 and 6 September 1997, respectively, regardless of the filing date of the international application. (See second paragraph above.)

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

CONFIRMATION OF PRECAUTIONARY DESIGNATIONS

This notification lists only specific designations made under Rule 4.9(a) in the request. It is important to check that these designations are correct. Errors in designations can be corrected where precautionary designations have been made under Rule 4.9(b). The applicant is hereby reminded that any precautionary designations may be confirmed according to Rule 4.9(c) before the expiration of 15 months from the priority date. If it is not confirmed, it will automatically be regarded as withdrawn by the applicant. There will be no reminder and no invitation. Confirmation of a designation consists of the filing of a notice specifying the designated State concerned (with an indication of the kind of protection or treatment desired) and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.

REQUIREMENTS REGARDING PRIORITY DOCUMENTS

For applicants who have not yet complied with the requirements regarding priority documents, the following is recalled.

Where the priority of an earlier national, regional or international application is claimed, the applicant must submit a copy of the said earlier application, certified by the authority with which it was filed ("the priority document") to the receiving Office (which will transmit it to the International Bureau) or directly to the International Bureau, before the expiration of 16 months from the priority date, provided that any such priority document may still be submitted to the International Bureau before that date of international publication of the international application, in which case that document will be considered to have been received by the International Bureau on the last day of the 16-month time limit (Rule 17.1(a)).

Where the priority document is issued by the receiving Office, the applicant may, instead of submitting the priority document, request the receiving Office to prepare and transmit the priority document to the International Bureau. Such request must be made before the expiration of the 16-month time limit and may be subjected by the receiving Office to the payment of a fee (Rule 17.1(b)).

If the priority document concerned is not submitted to the International Bureau or if the request to the receiving Office to prepare and transmit the priority document has not been made (and the corresponding fee, if any, paid) within the applicable time limit indicated under the preceding paragraphs, any designated State may disregard the priority claim, provided that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity to furnish the priority document within a time limit which is reasonable under the circumstances.

Where several priorities are claimed, the priority date to be considered for the purposes of computing the 16-month time limit is the filing date of the earliest application whose priority is claimed.



PATENT COOPERATION TREATY

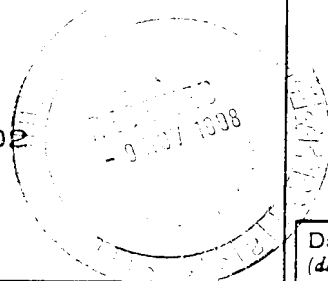
09/719533

From the RECEIVING OFFICE

PCT

To:

Drew & Napier
Robinson Road
P O Box 152
Singapore 900302



NOTIFICATION REGARDING CERTAIN
CORRECTIONS MADE *EX OFFICIO*

(PCT Administrative Instructions, Section 327)

Date of mailing
(day/month/year)

6 NOV 1998

Applicant's or agent's file reference

PCT/SG/13374

REPLY DUE

NONE

However, see paragraph 3 below

International application No.

PCT/SG 93/00045

International filing date

(day/month/year) 19 JUNE 1995 (19.06.95)

Applicant

GOVERNMENT OF THE REPUBLIC OF SINGAPORE

1. The applicant is hereby notified that this receiving Office has corrected formal defects in the international application *ex officio*, as shown on the attached copy of:



the request, sheet No.: 1



the description, sheet No.: _____



the claims, sheet No.: _____



the drawings, sheet No.: _____



other (specify): _____

2. If the applicant agrees with these corrections, no further action is required in this regard.
3. In case of disagreement with these corrections, the applicant should promptly inform this receiving Office accordingly.

Name and mailing address of the receiving Office

Registry of Patents

51 Bras Basah Road #04-01
Plaza By The Park

Facsimile No.

Singapore 0718

Fax No: 339-0252

Tel No: 330-2751

Authorized officer

MDM MISLIA BTE BUKHON

Registry of Patents

Tel: 330-2751

Telephone No.



PCT

HOME COPY

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

PCT/SG 98 / 00045

International Application No.

19 JUNE 1998 (19.06.98)

International Filing Date

REGISTRY OF PATENTS (SINGAPORE)

PCT INTERNATIONAL APPLICATION

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference

(if desired) (12 characters maximum)

PCT/8013374

Box No. I TITLE OF INVENTION

A VACCINE-INDUCED HEPATITIS B VIRAL STRAIN AND USES THEREOF

Box No. II APPLICANT

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)

GOVERNMENT OF THE REPUBLIC OF SINGAPORE

MINISTRY OF HEALTH
COLLEGE OF MEDICINE BUILDING
18 COLLEGE ROAD
SINGAPORE 169854

☐ This person is also inventor.

Telephone No.

(65) 325 9079

Facsimile No.

(65) 325 9211

Teleprinter No.

State (i.e. country) of nationality:

[N.A.] SG

State (i.e. country) of residence:

[N.A.] SG

This person is applicant for the purposes of:

☒ all designated States

☒ all designated States except the United States of America

☐ the United States of America only

☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)

DR CON CHONG JIN

14A PRINCESS OF WALES ROAD
SINGAPORE 266 914

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only (If this check-box is marked, do not fill in below.)

State (i.e. country) of nationality:

SINGAPOREAN

State (i.e. country) of residence:

SINGAPORE

This person is applicant for the purposes of:

☐ all designated States

☐ all designated States except the United States of America

☒ the United States of America only

☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:

☒ agent

☐ common representative

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

DREW & NAPIER

20 RAFFLES PLACE
#17-00 OCEAN TOWERS
SINGAPORE 048620

Telephone No.

(65) 535 0733

Facsimile No.

(65) 535 4906

Teleprinter No.

(65) 5330694

☐ Mark this check-box where no agent or common representative has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.



PATENT COOPERATION TREATY

199 / 719533

From the INTERNATIONAL BUREAU

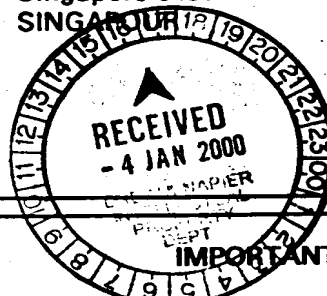
PCT

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

To:

DREW & NAPIER
20 Raffles Place
#17-00 Ocean Towers
Singapore 048620
SINGAPORE



| | | | |
|---|---|--|--|
| Date of mailing (day/month/year) 23 December 1999 (23.12.99) | | Applicant's or agent's file reference PCT/8013374 | |
| International application No. PCT/SG98/00045 | International filing date (day/month/year) 19 June 1998 (19.06.98) | Priority date (day/month/year) | |
| Applicant GOVERNMENT OF THE REPUBLIC OF SINGAPORE et al | | | |

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
AU,CN,EP,IL,JP,KP,KR,US

In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
AL,AM,AP,AT,AZ,BA,BB,BG,BR,BY,CA,CH,CU,CZ,DE,DK,EA,EE,ES,FI,GB,GE,GH,GM,GW,HU,ID,IS,KE,KG,KZ,LC,LK,LR,LS,LT,LU,LV,MD,MG,MK,MN,MW,MX,NO,NZ,OA,PL,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TR,TT,UA,UG,UZ,VN,YU,ZW
The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on 23 December 1999 (23.12.99) under No. WO 99/66047

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, a demand for international preliminary examination must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the national phase, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

| | |
|---|------------------------------------|
| The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland | Authorized officer J. Zahra |
| Facsimile No. (41-22) 740.14.35 | Telephone No. (41-22) 338.83.38 |

[illegible]

...and the fact that the *Journal* is a journal of the American Psychological Association, the largest and most influential of the professional organizations in the field of psychology, is a source of great strength and authority for the *Journal*.

Figure 1. The effect of the concentration of the *Agrobacterium* suspension on the transformation efficiency of *Agrobacterium* strains. The *Agrobacterium* strains were grown in the YEA medium for 24 h at 28 °C. The cell concentration of the strains was adjusted to 10⁸ cells/ml. The cell suspension was then mixed with the plant tissue and the transformation efficiency was determined. The results are shown as the mean ± SD of three independent experiments. The asterisk indicates a significant difference between the two strains (*p* < 0.05).

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Journal of Management Education 30(6)p.789-804

1997, 1998, 1999, 2000, 2001, 2002, 2003, 2004, 2005, 2006, 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2014, 2015, 2016, 2017, 2018, 2019, 2020, 2021, 2022, 2023, 2024, 2025, 2026, 2027, 2028, 2029, 2030, 2031, 2032, 2033, 2034, 2035, 2036, 2037, 2038, 2039, 2040, 2041, 2042, 2043, 2044, 2045, 2046, 2047, 2048, 2049, 2050, 2051, 2052, 2053, 2054, 2055, 2056, 2057, 2058, 2059, 2060, 2061, 2062, 2063, 2064, 2065, 2066, 2067, 2068, 2069, 2070, 2071, 2072, 2073, 2074, 2075, 2076, 2077, 2078, 2079, 2080, 2081, 2082, 2083, 2084, 2085, 2086, 2087, 2088, 2089, 2090, 2091, 2092, 2093, 2094, 2095, 2096, 2097, 2098, 2099, 2100, 2101, 2102, 2103, 2104, 2105, 2106, 2107, 2108, 2109, 2110, 2111, 2112, 2113, 2114, 2115, 2116, 2117, 2118, 2119, 2120, 2121, 2122, 2123, 2124, 2125, 2126, 2127, 2128, 2129, 2130, 2131, 2132, 2133, 2134, 2135, 2136, 2137, 2138, 2139, 2140, 2141, 2142, 2143, 2144, 2145, 2146, 2147, 2148, 2149, 2150, 2151, 2152, 2153, 2154, 2155, 2156, 2157, 2158, 2159, 2160, 2161, 2162, 2163, 2164, 2165, 2166, 2167, 2168, 2169, 2170, 2171, 2172, 2173, 2174, 2175, 2176, 2177, 2178, 2179, 2180, 2181, 2182, 2183, 2184, 2185, 2186, 2187, 2188, 2189, 2190, 2191, 2192, 2193, 2194, 2195, 2196, 2197, 2198, 2199, 2200, 2201, 2202, 2203, 2204, 2205, 2206, 2207, 2208, 2209, 2210, 2211, 2212, 2213, 2214, 2215, 2216, 2217, 2218, 2219, 2220, 2221, 2222, 2223, 2224, 2225, 2226, 2227, 2228, 2229, 2230, 2231, 2232, 2233, 2234, 2235, 2236, 2237, 2238, 2239, 2240, 2241, 2242, 2243, 2244, 2245, 2246, 2247, 2248, 2249, 2250, 2251, 2252, 2253, 2254, 2255, 2256, 2257, 2258, 2259, 2260, 2261, 2262, 2263, 2264, 2265, 2266, 2267, 2268, 2269, 2270, 2271, 2272, 2273, 2274, 2275, 2276, 2277, 2278, 2279, 2280, 2281, 2282, 2283, 2284, 2285, 2286, 2287, 2288, 2289, 2290, 2291, 2292, 2293, 2294, 2295, 2296, 2297, 2298, 2299, 2300, 2301, 2302, 2303, 2304, 2305, 2306, 2307, 2308, 2309, 2310, 2311, 2312, 2313, 2314, 2315, 2316, 2317, 2318, 2319, 2320, 2321, 2322, 2323, 2324, 2325, 2326, 2327, 2328, 2329, 2330, 2331, 2332, 2333, 2334, 2335, 2336, 2337, 2338, 2339, 2340, 2341, 2342, 2343, 2344, 2345, 2346, 2347, 2348, 2349, 2350, 2351, 2352, 2353, 2354, 2355, 2356, 2357, 2358, 2359, 2360, 2361, 2362, 2363, 2364, 2365, 2366, 2367, 2368, 2369, 2370, 2371, 2372, 2373, 2374, 2375, 2376, 2377, 2378, 2379, 2380, 2381, 2382, 2383, 2384, 2385, 2386, 2387, 2388, 2389, 2390, 2391, 2392, 2393, 2394, 2395, 2396, 2397, 2398, 2399, 2400, 2401, 2402, 2403, 2404, 2405, 2406, 2407, 2408, 2409, 2410, 2411, 2412, 2413, 2414, 2415, 2416, 2417, 2418, 2419, 2420, 2421, 2422, 2423, 2424, 2425, 2426, 2427, 2428, 2429, 2430, 2431, 2432, 2433, 2434, 2435, 2436, 2437, 2438, 2439, 2440, 2441, 2442, 2443, 2444, 2445, 2446, 2447, 2448, 2449, 2450, 2451, 2452, 2453, 2454, 2455, 2456, 2457, 2458, 2459, 2460, 2461, 2462, 2463, 2464, 2465, 2466, 2467, 2468, 2469, 2470, 2471, 2472, 2473, 2474, 2475, 2476, 2477, 2478, 2479, 2480, 2481, 2482, 2483, 2484, 2485, 2486, 2487, 2488, 2489, 2490, 2491, 2492, 2493, 2494, 2495, 2496, 2497, 2498, 2499, 2500, 2501, 2502, 2503, 2504, 2505, 2506, 2507, 2508, 2509, 2510, 2511, 2512, 2513, 2514, 2515, 2516, 2517, 2518, 2519, 2520, 2521, 2522, 2523, 2524, 2525, 2526, 2527, 2528, 2529, 2530, 2531, 2532, 2533, 2534, 2535, 2536, 2537, 2538, 2539, 2540, 2541, 2542, 2543, 2544, 2545, 2546, 2547, 2548, 2549, 2550, 2551, 2552, 2553, 2554, 2555, 2556, 2557, 2558, 2559, 2560, 2561, 2562, 2563, 2564, 2565, 2566, 2567, 2568, 2569, 2570, 2571, 2572, 2573, 2574, 2575, 2576, 2577, 2578, 2579, 2580, 2581, 2582, 2583, 2584, 2585, 2586, 2587, 2588, 2589, 2590, 2591, 2592, 2593, 2594, 2595, 2596, 2597, 2598, 2599, 2600, 2601, 2602, 2603, 2604, 2605, 2606, 2607, 2608, 2609, 2610, 2611, 2612, 2613, 2614, 2615, 2616, 2617, 2618, 2619, 2620, 2621, 2622, 2623, 2624, 2625, 2626, 2627, 2628, 2629, 2630, 2631, 2632, 2633, 2634, 2635, 2636, 2637, 2638, 2639, 2640, 2641, 2642, 2643, 2644, 2645, 2646, 2647, 2648, 2649, 2650, 2651, 2652, 2653, 2654, 2655, 2656, 2657, 2658, 2659, 2660, 2661, 2662, 2663, 2664, 2665, 2666, 2667, 2668, 2669, 2670, 2671, 2672, 2673, 2674, 2675, 2676, 2677, 2678, 26

1. *Phragmites australis* (Cav.) Trin. ex Steud. (Common reed)
 2. *Scirpus atrovirens* (L.) Link. (Black bog rush)
 3. *Scirpus cespitosus* (L.) Link. (Bog rush)
 4. *Scirpus setaceus* (L.) Link. (Bog rush)
 5. *Scirpus tabernaemontani* (L.) Link. (Bog rush)
 6. *Scirpus torreyana* (L.) Link. (Bog rush)
 7. *Scirpus validus* (L.) Link. (Bog rush)
 8. *Scirpus yagara* (L.) Link. (Bog rush)
 9. *Scirpus yagara* (L.) Link. (Bog rush)
 10. *Scirpus yagara* (L.) Link. (Bog rush)

PATENT COOPERATION TREATY

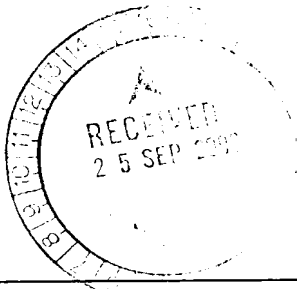
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From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

To:

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NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing
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15. 09. 00

Applicant's or agent's file reference
CG/PHM/PAT/8013374

IMPORTANT NOTIFICATION

International application No.
PCT/SG98/00045

International filing date (day/month/year)
19/06/1998

Priority date (day/month/year)
19/06/1998

Applicant
GOVERNMENT OF REPUBLIC OF SINGAPORE ... et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.
2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.
3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

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PATENT COOPERATION TREATY
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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

| | | | |
|---|---|--|--|
| Applicant's or agent's file reference CG/PHM/PAT/8013374 | | FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) | |
| International application No. PCT/SG98/00045 | International filing date (day/month/year) 19/06/1998 | Priority date (day/month/year) 19/06/1998 | |
| International Patent Classification (IPC) or national classification and IPC C12N15/51 | | | |
| Applicant GOVERNMENT OF REPUBLIC OF SINGAPORE ... et al. | | | |
| <p>1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 6 sheets, including this cover sheet.</p> <p><input checked="" type="checkbox"/> This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).</p> <p>These annexes consist of a total of 17 sheets.</p> | | | |
| <p>3. This report contains indications relating to the following items:</p> <ul style="list-style-type: none">I <input checked="" type="checkbox"/> Basis of the reportII <input type="checkbox"/> PriorityIII <input type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicabilityIV <input type="checkbox"/> Lack of unity of inventionV <input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statementVI <input type="checkbox"/> Certain documents citedVII <input checked="" type="checkbox"/> Certain defects in the international applicationVIII <input checked="" type="checkbox"/> Certain observations on the international application | | | |
| Date of submission of the demand 18/01/2000 | | Date of completion of this report 15. 09. 00 | |
| Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465 | | Authorized officer SCHEFFZYK, I Telephone No. +49 89 2399 8602  | |



**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/SG98/00045

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-35 as originally filed

Claims, No.:

1-74 as received on 13/06/2000 with letter of 09/06/2000

Drawings, sheets:

1/2,2/2 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:

☐ the claims, Nos.:

☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:



**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

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V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

| | | | |
|-------------------------------|------|--------|-----------------------------|
| Novelty (N) | Yes: | Claims | 1-26,35-74 |
| | No: | Claims | 27-34 |
| Inventive step (IS) | Yes: | Claims | 1-26,35-74 |
| | No: | Claims | 27-34 |
| Industrial applicability (IA) | Yes: | Claims | 1-50,55-74 |
| | No: | Claims | 51-54: see section VIII/8). |

2. Citations and explanations

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet



SECTION V-----

The subject-matter of claims 1-26, 35-74 is deemed novel and inventive since the existence of the Hepatitis B virus strain according to claim 1 which is characterized in that the surface major antigen contains at amino acid position 145 arginine in place of glycine was neither described nor derivable from the available prior art.

Thus, these claims meet the requirements of Art. 33(2)(3) PCT.

However, for the reasons set out below in section VIII(2), claims 27-34 cannot be considered to be novel over readily available antibodies directed against Hepatitis B viruses and methods of producing them.

Correspondingly, these claims do not comply with the requirements of Art. 33(2)(3) PCT.

SECTION VII-----

- 1). With respect to the expression "incorporated by reference" written in the specification Applicant's attention is drawn to Guidelines C-II 4.3 and C-II 4.18 PCT.

SECTION VIII-----

- 1). For the sake of clarity claim 1 should contain a reference to a SEQ.ID.NO.
- 2). With respect to claims 27-32 it is noted that it is questionable whether the methods defined in these claims actually contain all features essential to obtain antibodies exclusively binding to the mutated major surface antigen of hepatitis B virus which only differs from the corresponding wild-type protein in that it has arginine at position 145 instead of glycine. Relating to this it is noted that step e) defined in claims 27 and 31 also covers the detection and correspondingly also the provision of antibodies binding to regions of the wild-type major surface antigen! Moreover, the residues defined in claims 29 and 31 correspond to those of the wild-type major surface antigen.



**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/SG98/00045

- 3). The scope of claims 45, 46, 65, 66 is unclear since it is not known whether said claims are directed to a method of identifying a chemical compound or whether they are directed to the use of chemical compounds in a method for the manufacture of a medicament.
- 4). The term "derived from" used in claim 57 renders the scope of said claim unclear (Art. 6 PCT).
- 5). Claims 49, 50, 67 and 68 are not supported by the specification since the specification fails to mention any chemical compound identified by the method according to claims 45, 46, 65 and 66 (Art. 6 PCT in combination with Guidelines C-III 6.3 PCT). Moreover, with respect to these claims an objection under Art. 5 PCT also arises since in the absence of evidence showing that chemical compounds suitable to exclusively bind to the mutant major surface antigen having at position 145 arginine in place of glycine actually exist the existence of such compounds is merely speculative and thus it is questionable whether a person skilled in the art actually is able to prepare the claimed compositions.
- 6). The same objections apply equally to claims 52 and 46 relating to the use of the compositions according to claims 49, 50, 67 and 68.
- 7). The reference in claims 52, 54, 70 and 72 should be checked (claims 45, 46 and 67 are not directed to compositions but to methods).
- 8). Claims 51-54, 57-64, 69-72 are not supported by the specification since the application as originally filed fails to provide any facts and data demonstrating that the mutant form (Arg instead of Gly at position 145) of the major surface antigen is actually suitable for the medical applications defined in these claims. Relating to this it is also pointed out that there is no evidence in the application showing a relationship of the occurrence of said mutant form with the development of HCC (hepatocellular carcinoma).
- 9). Claims 51-54 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these



**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

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claims (Article 34(4)(a)(i) PCT).



What is claimed is:

1. An isolated strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) and deposited under Accession Nos. P97121501, P97121502 and P97121503 with the European Collection of Cell Culture on 15th December 1997.
2. An isolated nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine.
3. The isolated nucleic acid of claim 2, wherein the polypeptide is being encoded by nucleotides 155 through 835 of the nucleic acid sequence designated SEQ. I.D. No. 1.
4. The isolated nucleic acid of claim 3, comprising nucleotides "AGA" in positions 587-589.
5. The isolated nucleic acid of claim 2, wherein the nucleic acid is DNA.
6. The isolated nucleic acid of claim 2, wherein the nucleic acid is RNA.
7. The isolated nucleic acid of claim 5, wherein the nucleic acid is cDNA.
8. The isolated nucleic acid of claim 5, wherein the nucleic acid is genomic DNA.
9. The isolated nucleic acid of claim 2, wherein the polypeptide

AMENDED SHEET



has an amino acid sequence substantially the same as amino acid residues 174 through 400 of the amino acid sequence designated SEQ. I.D. No. 3.

10. An isolated nucleic acid which encodes a peptide, wherein the peptide is encoded by a nucleic acid molecule comprising nucleotides 527 through 595 of SEQ. I.D. No. 1.
11. An isolated nucleic acid which encodes a peptide, wherein the peptide has an amino acid sequence comprising amino acid residues 298 through 320 of the amino acid sequence designated SEQ. I.D. No. 3.
12. A vector comprising an isolated nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine and operatively linked to a promoter of RNA transcription.
13. A vector comprising an isolated nucleic acid encoding a peptide, wherein the peptide is encoded by a nucleic acid molecule comprising nucleotides 527 through 595 of SEQ. I.D. No. 1.
14. The vector of claim 12 or 13, wherein the vector comprises viral DNA.
15. A host vector system for the production of a polypeptide which comprises the vector of claim 12 in a suitable host.
16. A host vector system for the production of a peptide which comprises the vector of claim 13 in a suitable host.

AMENDED SHEET



17. A method of producing a polypeptide which comprises growing the host vector system of claim 15 under suitable conditions permitting production of the polypeptide and recovering the polypeptide so produced.
18. A method of producing a peptide which comprises growing the host vector system of claim 16 under suitable conditions permitting production of the polypeptide and recovering the polypeptide so produced.
19. A method of obtaining a polypeptide in purified form which comprises:
 - (a) introducing the vector of claim 12 into a suitable host cell;
 - (b) culturing the resulting host cell so as to produce the polypeptide;
 - (c) recovering the polypeptide produced in step (b); and
 - (d) purifying the polypeptide so recovered.
20. A method of obtaining a polypeptide in purified form which comprises:
 - (a) introducing the vector of claim 13 into a suitable host cell;
 - (b) culturing the resulting host cell so as to produce the polypeptide;
 - (c) recovering the polypeptide produced in step (b); and
 - (d) purifying the polypeptide so recovered.
21. A purified polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus deposited under Accession Nos. P97121501, P97121502 and P97121503 with the European Collection of Cell Culture on 15th December 1997, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine.

AMENDED SHEET



22. A purified polypeptide obtained from a method which comprises:
- (a) introducing a vector comprising an isolated nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus deposited under Accession Nos. P97121501, P97121502 and P97121503 with the European Collection of Cell Culture on 15th December 1997, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine and operatively linked to a promoter of RNA transcription into a suitable host cell;
 - (b) culturing the resulting host cell so as to produce the polypeptide;
 - (c) recovering the polypeptide produced in step (b) ; and
 - (d) purifying the polypeptide so recovered.
23. A purified peptide, wherein the peptide has an amino acid sequence comprising amino acid residues 298 through 320 of the amino acid sequence designated SEQ. I.D. No. 3.
24. A purified peptide obtained from a method which comprises:
- (a) introducing a vector comprising an isolated nuclei acid encoding a peptide which comprises at least a portion of a mutant major surface antigen of a strain of hepatitis B virus deposited under Accession Nos. P97121501, P97121502 and P97121503 with the European Collection of Cell Culture on 15th December 1997 wherein the peptide is encoded by a nucleic acid molecule comprising nucleotides 527 through 595 of SEQ. I.D. No. 1 into a suitable host cell;
 - (b) culturing the resulting host cell so as to produce the polypeptide;
 - (c) recovering the polypeptide produced in step (b); and



- (d) purifying the polypeptide so recovered.
25. An oligonucleotide of at least 15 nucleotides capable of specifically hybridizing with a unique sequence of nucleotides within a nucleic acid which encodes a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine, without hybridizing to any sequence of nucleotides within a nucleic acid which encodes the major surface antigen of a wildtype hepatitis B virus.
26. The oligonucleotide of claim 25 comprising nucleotides 527 through 595 of SEQ. I.D. No. 1.
27. A method of obtaining antibodies to a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and not to the major surface antigen of a wildtype hepatitis B virus, comprising:
- (a) obtaining the polypeptide in a purified form;
 - (b) immunizing an organism capable of producing antibodies against the purified polypeptide;
 - (c) collecting the produced antibodies;
 - (d) combining the produced antibodies and the purified polypeptide under conditions to form a complex; and
 - (e) determining which produced antibodies form a complex with the purified polypeptide so as to obtain antibodies to the polypeptide.



28. The method of claim 27, wherein the polypeptide is being encoded by nucleotides 155 through 835 of the nucleic acid sequence designated SEQ. I.D. No. 1.
29. The method of claim 27, wherein the polypeptide has an amino acid sequence substantially the same as amino acid residues 174 through 400 of the amino acid sequence designated SEQ. I.D. No. 3.
30. The method of claim 27, wherein the organism comprises a rabbit or a mouse.
31. A method of obtaining antibodies to a peptide, wherein the peptide has an amino acid sequence comprising amino acid residues 298 through 320 of the amino acid sequence designated SEQ. I.D. No. 3, comprising:
 - (a) obtaining the peptide in a purified form;
 - (b) immunizing an organism capable of producing antibodies against the purified peptide;
 - (c) collecting the produced antibodies;
 - (d) combining the produced antibodies and the purified peptide under conditions to form a complex; and
 - (e) determining which produced antibodies form a complex with the purified peptide so as to obtain antibodies to the peptide.
32. The method of claim 31, wherein the organism comprises a rabbit or a mouse.
33. The antibodies obtained in claim 27 or 31.
34. Monoclonal antibodies of the antibodies of claim 33.
35. Antibodies capable of detecting a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus,



such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and incapable of detecting the major surface antigen of a wildtype hepatitis B virus.

36. Antibodies capable of detecting a peptide, wherein the peptide has an amino acid sequence comprising amino acid residues 298 through 320 of the amino acid sequence designated SEQ. I.D. No. 3.
37. Use of a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine for determining whether a subject is infected with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine), wherein such determination comprises
 - (a) obtaining an appropriate nucleic acid sample from the subject; and
 - (b) determining whether the nucleic acid sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine.
38. The use of claim 37, wherein the nucleic acid sample in step



(a) comprises mRNA corresponding to the transcript of DNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and wherein the determining of step (b) comprises:

- (i) contacting the mRNA with the oligonucleotide of claim 25 under conditions permitting binding of the mRNA to the oligonucleotide so as to form a complex;
- (ii) isolating the complex so formed; and
- (iii) identifying the mRNA in the isolated complex so as to thereby determine whether the mRNA is, or is derived from, a nucleic acid which encodes the polypeptide.

39. The use of claim 37, wherein the nucleic acid sample in step (a) comprises mRNA corresponding to the transcript of DNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and wherein the determining of step (b) comprises:

- (i) translating the mRNA under suitable conditions to obtain an amino acid sequence; and
- (ii) comparing the amino acid sequence of step (i) with the amino acid sequence encoded by the isolated nucleic acid of claim 9 so as to determine whether the nucleic acid sample is, or is derived from, a nucleic acid which encodes the polypeptide.



40. The use of claim 37, wherein the determining of step (b) comprises:
- (i) amplifying the nucleic acid present in the sample of step (a); and
 - (ii) detecting the presence of polypeptide in the resulting amplified nucleic acid.
41. Use of antibodies capable of detecting a polypeptide which is a mutant major surface antigen of a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) for determining whether a subject is infected with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine), wherein such determination comprises:
- (a) obtaining an appropriate sample from the subject; and
 - (b) determining whether the sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, by contacting the sample under appropriate conditions to bind to the antibodies of claim 35 or 36 so as to determine whether a subject is infected.
42. The use of claim 37, 38 or 41, wherein the isolated nucleic acid, oligonucleotide, or antibody is labeled with a detectable marker.
43. The use the claim 42, wherein the detectable marker is a radioactive isotope, a fluorophor, or an enzyme.



44. The use of claim 37, wherein the sample comprises blood, tissue, or sera.
45. A method for identifying a chemical compound for use in the manufacture of a medicament capable of treating infection by a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine), wherein the method for identifying the chemical compound comprises:
- (a) contacting a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, with the chemical compound under conditions permitting binding between the polypeptide and the chemical compound;
 - (b) detecting specific binding of the chemical compound to the polypeptide; and
 - (c) determining whether the chemical compound binds to the polypeptide so as to identify a chemical compound which is capable of treating infection by the viral strain.
46. A method for identifying a chemical compound for use in the manufacture of a medicament capable of preventing infection by a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine), wherein the method for identifying the chemical compound comprises:
- (a) contacting a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen



of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, with the chemical compound under conditions permitting binding between the polypeptide and the chemical compound;

- (b) detecting specific binding of the chemical compound to the polypeptide; and
 - (c) determining whether the chemical compound binds to the polypeptide so as to identify a chemical compound which is capable of preventing infection by the viral strain.
47. A composition comprising a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus deposited under Accession Nos. P97121501, P97121502 and P97121503 with the European Collection of Cell Culture on 15th December 1997, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, the amounts of such polypeptide being effective to stimulate or enhance antibody production in a subject, and a pharmaceutically acceptable carrier.
48. A composition comprising a peptide, wherein the peptide has an amino acid sequence comprising amino acid residues 298 through 320 of the amino acid sequence designated SEQ. I.D. No. 3., the amounts of such peptide being effective to stimulate or enhance antibody production in a subject, and a pharmaceutically acceptable carrier.
49. A method of obtaining a composition which comprises a identifying a chemical compound by the method of claim 45 and admixing the compound with a pharmaceutically effective carrier.



50. A method of obtaining a composition which comprises a identifying a chemical compound by the method of claim 46 and admixing the compound with a pharmaceutically effective carrier.
51. Use of the composition of claim 47 or 48 for treating a subject infected with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine).
52. Use of the composition of claim 49 for treating a subject infected with a strain of hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine).
53. Use of the composition of claim 47 or 48 for preventing infection by a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) in a subject.
54. Use of the composition of claim 50 for preventing infection with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) in a subject.
55. A method of screening bodily fluids from a subject for a strain of hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) which comprises:
 - (a) obtaining an appropriate sample of bodily fluid from the subject;
 - (b) determining the presence of a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major



surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, in the sample of step (a) so as to screen the sample for the strain.

56. The method of claim 55, wherein the bodily fluid comprises blood, sera, or a nucleic acid sample of blood or sera.
57. Use of an antibody that recognizes a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus for determining whether the subject has a predisposition for hepatocellular carcinoma, wherein such determination comprises:
 - (a) obtaining an appropriate nucleic acid sample from the subject; and
 - (b) determining whether the nucleic acid sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, by contacting the sample under appropriate conditions to bind to the antibodies of claim 35 so as to determine whether the subject has a predisposition for hepatocellular carcinoma.
58. The method of claim 57, wherein the nucleic acid sample in step (a) comprises mRNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a



glycine, and wherein the determining of step (b) comprises:

- (i) contacting the mRNA with the oligonucleotide of claim 25 under conditions permitting binding of the mRNA to the oligonucleotide so as to form a complex;
- (ii) isolating the complex so formed; and
- (iii) identifying the mRNA in the isolated complex so as to thereby determine whether the mRNA is, or is derived from, a nucleic acid which encodes the polypeptide.

59. The method of claim 57, wherein the nucleic acid sample in step (a) comprises mRNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and wherein the determining of step (b) comprises:

- (i) translating the mRNA under suitable conditions to obtain an amino acid sequence; and
- (ii) comparing the amino acid sequence of step (i) with the amino acid sequence encoded by the isolated nucleic acid of claim 9 so as to determine whether the nucleic acid sample is, or is derived from, a nucleic acid which encodes the polypeptide.

60. The method of claim 57, wherein the determining of step (b) comprises:

- (i) amplifying the nucleic acid present in the sample of step (a); and
- (ii) detecting the presence of polypeptide in the resulting amplified nucleic acid.

61. Use of an antibody that recognizes a polypeptide which is a

AMENDED SHEET



mutant major surface antigen of a strain of hepatitis B virus for determining whether the subject has a predisposition for hepatocellular carcinoma, wherein such determination comprises:

- (a) obtaining an appropriate sample from the subject; and
 - (b) determining whether the sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, by contacting the sample under appropriate conditions to bind to the antibodies of claim 36 so as to determine whether the subject has a predisposition for hepatocellular carcinoma.
62. The method of claim 58, 59 or 60, wherein the oligonucleotide or antibody is labeled with a detectable marker.
63. The method of claim 62, wherein the detectable marker is a radioactive isotope, a fluorophor or an enzyme.
64. The method of claim 57, wherein the sample comprises blood, tissue or sera.
65. A method for identifying a chemical compound for use in the manufacture of a medicament capable of treating hepatocellular carcinoma wherein the method for identifying the chemical compound comprises:
- (a) contacting a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen



of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, with the chemical compound under conditions permitting binding between the polypeptide and the chemical compound;

- (b) detecting specific binding of the chemical compound to the polypeptide; and
- (c) determining whether the chemical compound binds to the polypeptide so as to identify a chemical compound which is capable of treating hepatocellular carcinoma.

66. A method for identifying a chemical compound for use in the manufacture of a medicament capable of preventing hepatocellular carcinoma, wherein the method for identifying the chemical compound comprises:

- (a) contacting a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, with the chemical compound under conditions permitting binding between the polypeptide and the chemical compound;
- (b) detecting specific binding of the chemical compound to the polypeptide; and
- (c) determining whether the chemical compound binds to the polypeptide so as to identify a chemical compound which is capable of preventing hepatocellular carcinoma.

67. A method of obtaining a composition which comprises a identifying a chemical compound by the method of claim 65 and admixing the compound with a pharmaceutically effective carrier.



68. A method of obtaining a composition which comprises a identifying a chemical compound by the method of claim 66 and admixing the compound with a pharmaceutically effective carrier.
69. Use of the composition of claim 47 or 48 as a medicament for treating hepatocellular carcinoma.
70. Use of the composition of claim 67 as a medicament for treating hepatocellular carcinoma.
71. Use of the composition of claim 47 or 48 as a medicament for preventing hepatocellular carcinoma.
72. Use of the composition of claim 67 as a medicament for preventing hepatocellular carcinoma.
73. A hepatitis vaccine, comprising a mutant form of the surface antigen of hepatitis B virus deposited under Accession Nos. P97121501, P97121502 and P97121503 with the European Collection of Cell Culture on 15th December 1997, such polypeptide having an amino acid sequence which differs from the amino acid sequence of the major surface antigen of hepatitis B in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine.
74. The vaccine of claim 73, further comprising an adjuvant.

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09/719583

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

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International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference
(if desired) (12 characters maximum)

PCT/8013374

Box No. I TITLE OF INVENTION

A VACCINE-INDUCED HEPATITIS B VIRAL STRAIN AND USES THEREOF

Box No. II APPLICANT

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)

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☐ This person is also inventor.

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This person is applicant
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Statesall designated States except
the United States of Americathe United States
of America onlythe States indicated in
the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)

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☐ applicant only☒ applicant and inventor☐ inventor only (If this check-box
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State (i.e. country) of residence:

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This person is applicant
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Statesall designated States except
the United States of Americathe United States
of America onlythe States indicated in
the Supplemental Box☐ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:



agent



common representative

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

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Continuation of Box No. III FURTHER APPLICANTS AND/OR (FURTHER) INVENTORS

If none of the following sub-boxes is used, this sheet is not to be included in the request.

Name and address: (Family name followed by given name, for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)

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State (i.e. country) of residence:

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This person is applicant for the purposes of:

☐ all designated States☐ all designated States except the United States of America☒ the United States of America only☐ the States indicated in the Supplemental Box

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State (i.e. country) of residence:

SINGAPORE

This person is applicant for the purposes of:

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State (i.e. country) of residence:

SINGAPORE

This person is applicant for the purposes of:

☐ all designated States☐ all designated States except the United States of America☒ the United States of America only☐ the States indicated in the Supplemental Box

Name and address: (Family name followed by given name, for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)

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State (i.e. country) of residence:

SINGAPORE

This person is applicant for the purposes of:

☐ all designated States☐ all designated States except the United States of America☒ the United States of America only☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on another continuation sheet.



Box No. VI PRIORITY CLAIM Further priority claims are indicated in the Supplemental Box ☐

The priority of the following earlier application(s) is hereby claimed:

| Country in which or for which the application was filed | Filing Date (day/month/year) | Application No. | Office of filing only for regional or international application |
|---|---------------------------------|-----------------|---|
| Item 1: - | - | - | - |
| Item 2: | | | |
| Item 3: | | | |

Mark the following check-box if the certified copy of the earlier application is to be issued by the Office which (for the purposes of the present international application) is the receiving Office (a fee may be required)

☐ The receiving Office is hereby requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) identified above as item(s)

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) (If two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used).

ISA / AT

Earlier search: Fill in where a search (international, international-type or other) by the International Searching Authority has already been carried out or requested and the Authority is now requested to base the international search, to the extent possible, on the results of that earlier search. (Identify such search or request either by reference to the relevant application (or the translation thereof) or by reference to the search request.)

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Box No. VIII CHECK LIST

This international application contains the following number of sheets:

- | | | |
|----------------|----|--------|
| 1. request | 4 | sheets |
| 2. description | 34 | sheets |
| 3. claims | 18 | sheets |
| 4. abstract | 1 | sheets |
| 5. drawings | 13 | sheets |

Total : 70 sheets

This international application is accompanied by the item(s) marked below:


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| 1. <input type="checkbox"/> separate signed power of attorney | 5. <input checked="" type="checkbox"/> fee calculation sheet |
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Figure No. _____ of the drawings (if any) should accompany the abstract when it is published.

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).


CECELIA GIRVIN
DREW & NAPIER


JUPITER KONG
DREW & NAPIER

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The following designations are hereby made under Rule 4.9(a) (mark the applicable check-boxes: at least one must be marked):

Regional Patent

- ☒ AP ARIPO Patent: GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, SD Sudan, SZ Swaziland, UG Uganda, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT
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The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. (Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)



PCT

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REQUEST

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PCT/SG 98 / 00045

International Application No.

19 JUNE 1998 (19.06.98)

International Filing Date

REGISTRY OF PATENTS (SINGAPORE)
PCT INTERNATIONAL APPLICATION

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference

(if desired) (12 characters maximum) PCT/8013374

Box No. I TITLE OF INVENTION

A VACCINE-INDUCED HEPATITIS B VIRAL STRAIN AND USES THEREOF

Box No. II APPLICANT

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)

GOVERNMENT OF THE REPUBLIC OF SINGAPORE

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State (i.e. country) of residence:

[N.A.]^{▲▲} SG[▲]

This person is applicant for the purposes of:

☒ all designated States☐ all designated States except the United States of America☐ the United States of America only☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)

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This person is:

☐ applicant only☒ applicant and inventor☐ inventor only (If this check-box is marked, do not fill in below.)

State (i.e. country) of nationality:

SINGAPOREAN[▲]

State (i.e. country) of residence:

SINGAPORE

This person is applicant for the purposes of:

☐ all designated States☐ all designated States except the United States of America☒ the United States of America only☐ the States indicated in the Supplemental Box☐ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:

☒ agent☐ common representative

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

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PCT / 8013374

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International application No.

PCT ISG 98100045

International filing date

(day/month/year) 19 JUNE 1998 (19-06-98)

Applicant

GOVERNMENT OF THE REPUBLIC OF SINGAPORE

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the request, sheet No.:

1



the description, sheet No.:



the claims, sheet No.:



the drawings, sheet No.:



other (specify):

2. If the applicant agrees with these corrections, no further action is required in this regard.
3. In case of disagreement with these corrections, the applicant should promptly inform this receiving Office accordingly.

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